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SYNTHESIS AND ACID CATALYSED HYDROLYSIS OF ADENINE NUCLEOSIDES WITH AN ACYCLIC SUGAR

by

GORDON G. CROSS

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

IN

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EDMONTON, ALBERTA

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THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled SYNTHESIS AND ACID CATALYSED HYDROLYSIS OF ADENINE NUCLEOSIDES WITH AN ACYCLIC SUGAR submitted by Gordon G. Cross in partial fulfilment of the requirements for the degree of Master of Science in Chemistry.

ABSTRACT

Two series of adenine nucleosides were synthesized having the 2'-3' bond broken by periodate oxidation-borohydride reduction. One series was substituted at the original 2' position and the other at the original 5' position. These analogues have considerable conformational freedom and are potential antimetabolites. In addition, the effects of the intact sugar ring are not a factor in acid catalysed glycosyl bond hydrolysis.

The 2'-substituted series was prepared using ethoxymethylidene as a 3',5' cyclic blocking group. The 2'-O-tosyl derivative was prepared and then further transformations carried out at this position. The blocking group was removed under mild acidic conditions. A series of 5'-substituted adenosines was prepared, usually by routes different from those published in the literature. These were then converted to their acyclic analogues by oxidation-reduction.

The acid catalysed glycosyl bond hydrolysis of these compounds was studied in 1 M HCl in dioxane/water at 50°C. All compounds showed pseudo-first order kinetics, which is consistent with the A1 mechanism widely accepted for purine nucleoside hydrolysis. The much faster hydrolysis of the acyclic analogues is explained in terms of oxygen lone-pair stabilization of the carbonium ion which develops at C 1'. The order of acid stabilities of the 5'-substituted

compounds correlated with the electron withdrawing ability of the 5'-substituent but the large stabilizing effect of 5'-substituents was surprising. Within the 2'-substituted series both electronic and steric effects were important. 2'-Azido- and 2'-amino-2'-deoxyadenosine$_{ox-red}$ (48 and 55) showed unexpected acid lability which was not satisfactorily explained.

ACKNOWLEDGMENTS

I would like to thank Dr. Robins for his patient encouragement and advice, and the other members of the research group for much helpful advice and a good time.

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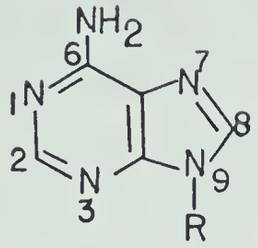
INTRODUCTION

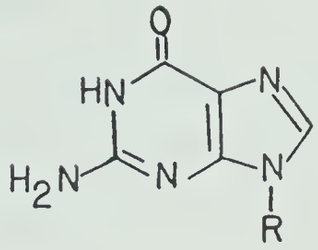
A BRIEF HISTORY OF NUCLEOSIDES.

Nucleic acids were first discovered in 1871 when Meischer isolated a material he called nuclein from the nuclei of pus cells.[1] Altmann subsequently introduced the term "nucleic acid" for this material and developed procedures for isolating nucleic acids from various sources.[2] The term "nucleoside" was first used by Levene and Jacobs to describe the carbohydrate derivatives of purines and pyrimidines which they isolated from alkaline hydrolysates of yeast nucleic acid.[3] This term is now used to refer to a carbohydrate or carbohydrate derivative linked to a heterocyclic base whether by a C-N or C-C bond. This includes both naturally occurring and synthetic compounds.

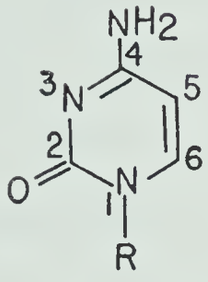
Nucleic acids are polymers of nucleosides joined by 3'-5' phosphodiester linkages. They fall into two general categories: ribonucleic acid (RNA) in which the carbohydrate is D-ribose and deoxyribonucleic acid (DNA) in which the carbohydrate is 2-deoxy-D-*erythro*-pentose(2-deoxy-D-ribose). The nucleosides commonly found in RNA are the purine ribonucleosides adenosine (1) and guanosine (2), and the pyrimidine ribonucleosides cytidine (3) and uridine (4). Those commonly occurring in DNA are 2'-deoxyadenosine (5), 2'-deoxyguanosine (6), 2'-deoxycytidine (7) and thymidine (8). In addition RNAs contain many minor



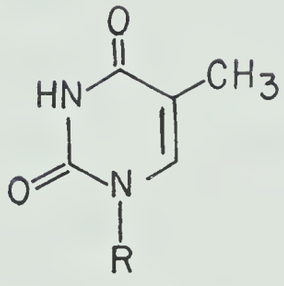
1 R=Rib
5 R=2'dRib
9 R=H



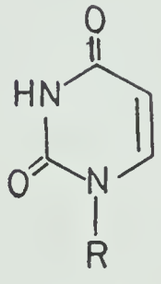
2 R=Rib
6 R=2'dRib
10 R=H



3 R=Rib
7 R=2'dRib
11 R=H

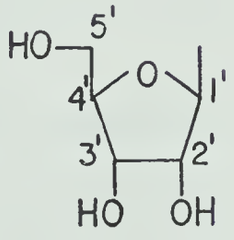


8 R=2'dRib
12 R=H

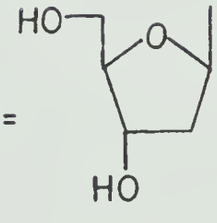


4 R=Rib
13 R=H

Rib=



2'dRib=

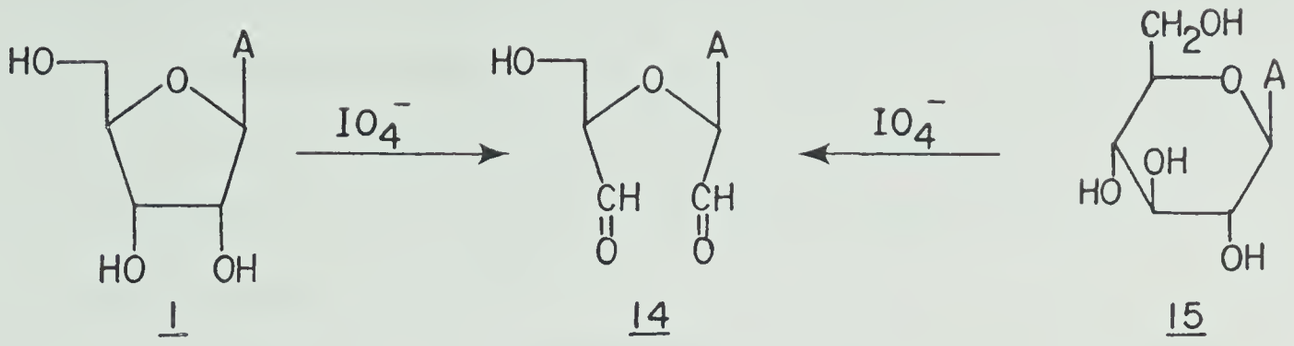


nucleosides which are quite often simple base-methylated derivatives of the common ribosides.[4,5]

Nucleosides were first hydrolysed into base and sugar components by Kossel in 1891[6] and shortly afterwards the structures of the common bases were established as adenine (9), guanine (10), cytosine (11), thymine (12), and uracil (13).[7]

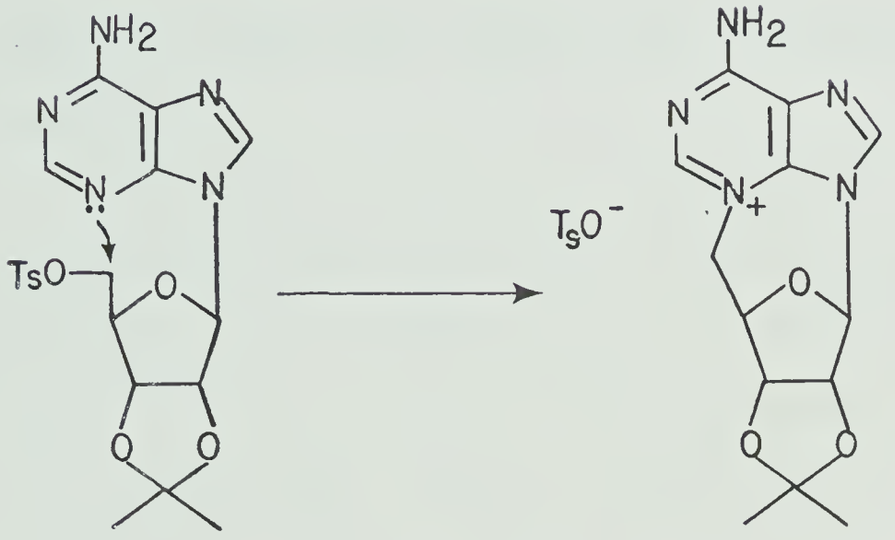
Levene and coworkers isolated the carbohydrate moeity of ribonucleic acid and established its identity as D-ribose,[8] and much later isolated and determined the structure of 2-deoxy-D-ribose from DNA.[9,10] The furanose ring size of the ribose was determined by the same group, by methylation of nucleosides followed by hydrolysis and identification of the sugar.[11] Levene and coworkers also established the positions of attachment of the sugar on the bases by chemical methods.[12,13] These assignments were confirmed by Gulland and coworkers by comparison with the ultraviolet spectra of specifically methylated bases.[14,15,16]

Periodate oxidation studies with adenosine by Todd and coworkers confirmed that the ribose was a furanoside, since it consumed one mole of periodate whereas a pyranoside would consume two. The configuration at the anomeric centre was established as β since the dialdehyde product obtained (14) was identical to that obtained by periodate oxidation of the β glucoside 15.[17] (Scheme I). Todd

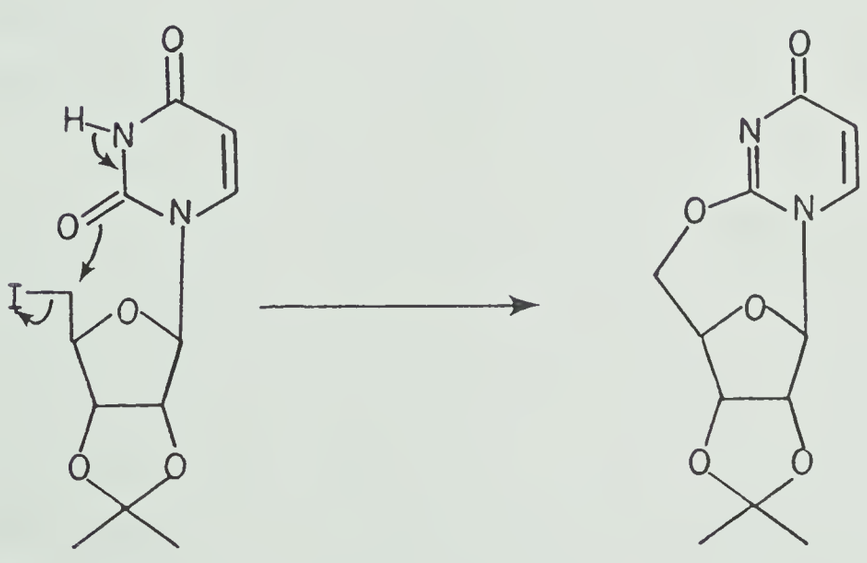


1 14 15

Scheme I



16 17



18 19

Scheme II

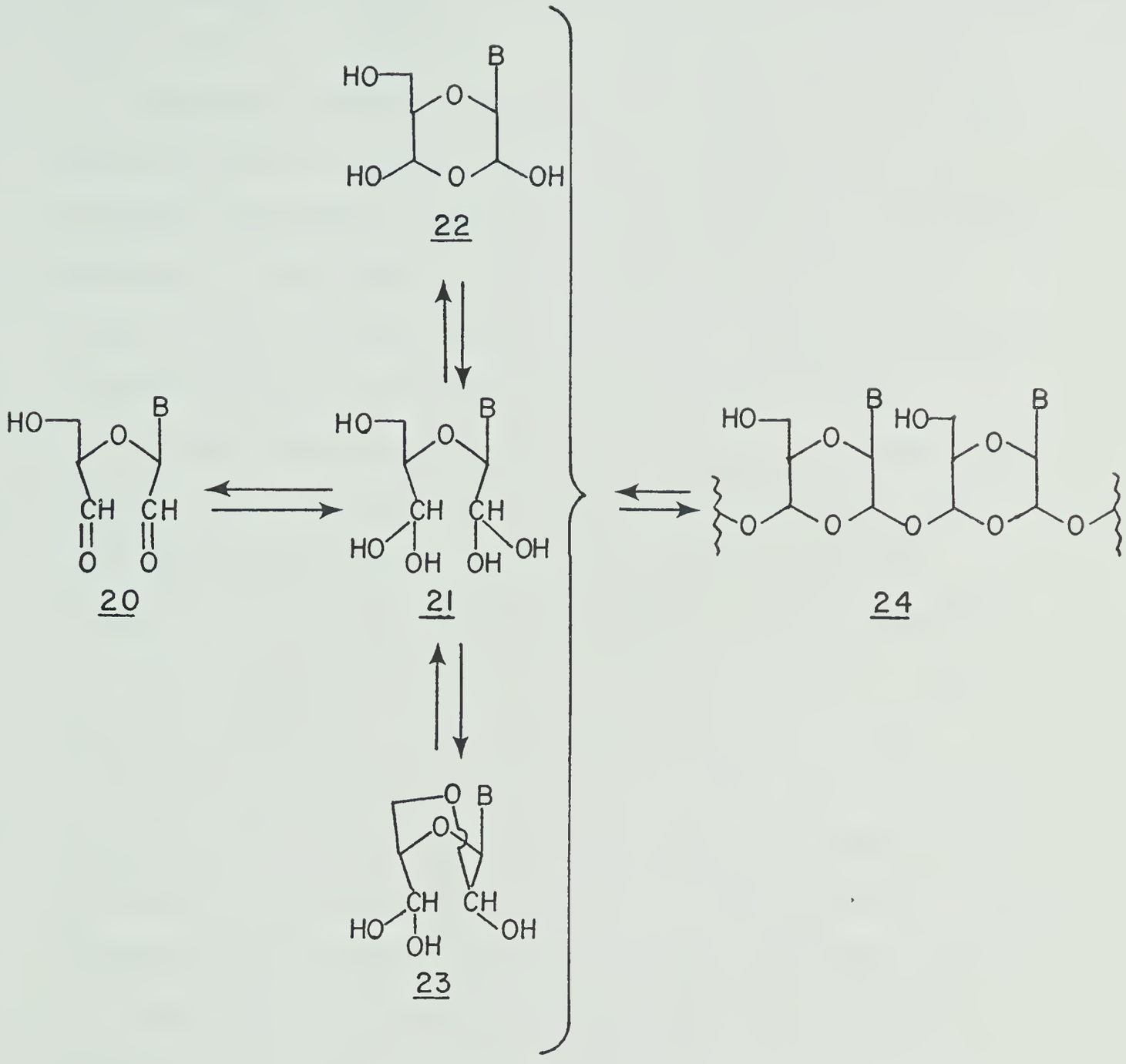
confirmed the β configuration by demonstrating the formation of cyclonucleosides 17 and 19,[18] which is possible only for the 1',4'-cis(β-D)nucleosides (Scheme II) and by total synthesis of the commonly occurring nucleosides via unambiguous routes.[19]

The foregoing material is the subject of several extensive reviews.[20-26]

SURVEY OF OPEN-CHAIN NUCLEOSIDES - OCCURRENCE, PROPERTIES AND USES.

As outlined above, the ring-opened "dialdehydes" of nucleosides resulting from periodate oxidation were first prepared in the course of determining nucleoside structure.[17,27] It was soon discovered that the dialdehydes of 5'-nucleotides readily undergo β-elimination of the 5'-phosphate group. Periodate oxidation in the presence of an alkyl amine, which will catalyse elimination, is used as a method of sequential degradation of ribonucleic acid fragments starting from the free 3'-hydroxyl end.[28-32]

The dialdehydes of common naturally occurring ribonucleosides were isolated and characterized only as various derivatives for some time.[17,27,33,34] In 1971 Cramer and coworkers reported that the dialdehyde of adenosine (14) could be crystallized from hot water[35] and Jones *et al.* then reported crystallization of the dialdehydes of all four common ribonucleosides.[36] Both

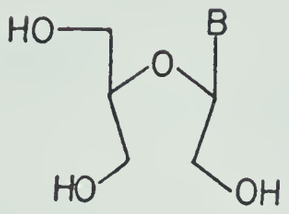

HO
B
O
HO
O
OH
22
HO
B
O
CH
CH
O
O
20
HO
B
O
CH
CH
HO
OH
OH
OH
21
HO
B
O
HO
B
O
O
O
O
O
O
24
O
B
O
CH
CH
HO
OH
OH
23

Scheme III

of these groups concluded from spectral evidence that only a small fraction of these molecules exist as the free aldehyde in aqueous solution; most existed as various hydrated species (21-24). The infrared spectra showed no aldehyde carbonyl or C-H stretch absorptions but did show a band at 1100 cm^{-1} which is characteristic of polymeric aldehydes such as paraformaldehyde. The peaks assigned to H2' and H3' in the PMR spectra were very broad and not as far downfield as free aldehyde protons. Peaks at δ 9-10 were very small if visible at all. Ultraviolet spectra were identical to those of the parent nucleosides. As would be expected due to the greater conformational flexibility of the opened ring, the magnitude of Cotton effects observed in the CD spectra of these compounds were greatly reduced except in the case of guanosine. The increased magnitude in the guanosine product was attributed to self-association of oligomers.[37] Jones concluded from the tlc behaviour and ORD spectra that in dilute solution the predominant species were hydrated monomers,[36] while Cramer concluded from IR and PMR spectra that these dialdehydes were completely polymeric (23) in the solid state.[37]

Borohydride reduction of the dialdehydes, in whatever hydrated form, generates the opened chain trialcohols 25-28. In 1970 Lerner prepared, purified, and characterized the oxidized-reduced analogues of adenosine (25),

cytidine (27) and uridine (28).[38] Khym and Cohn reported



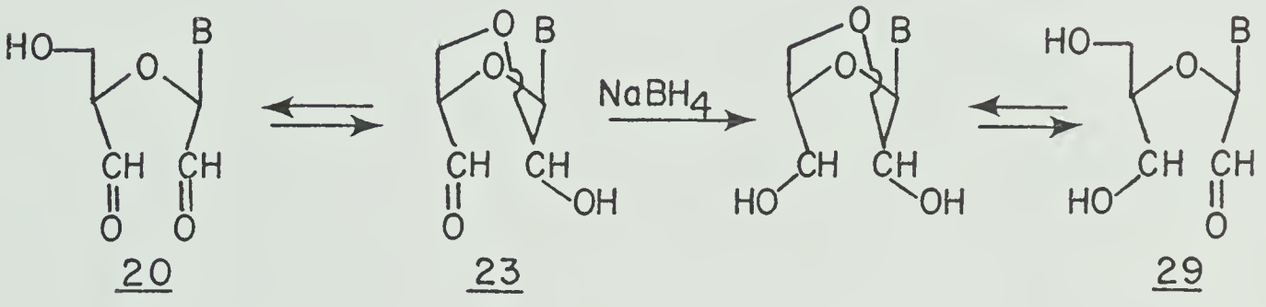
25 B = Adenin-9-yl

26 B = Guanin-9-yl

27 B = Cytosin-1-yl

28 B = Uracil-1-yl

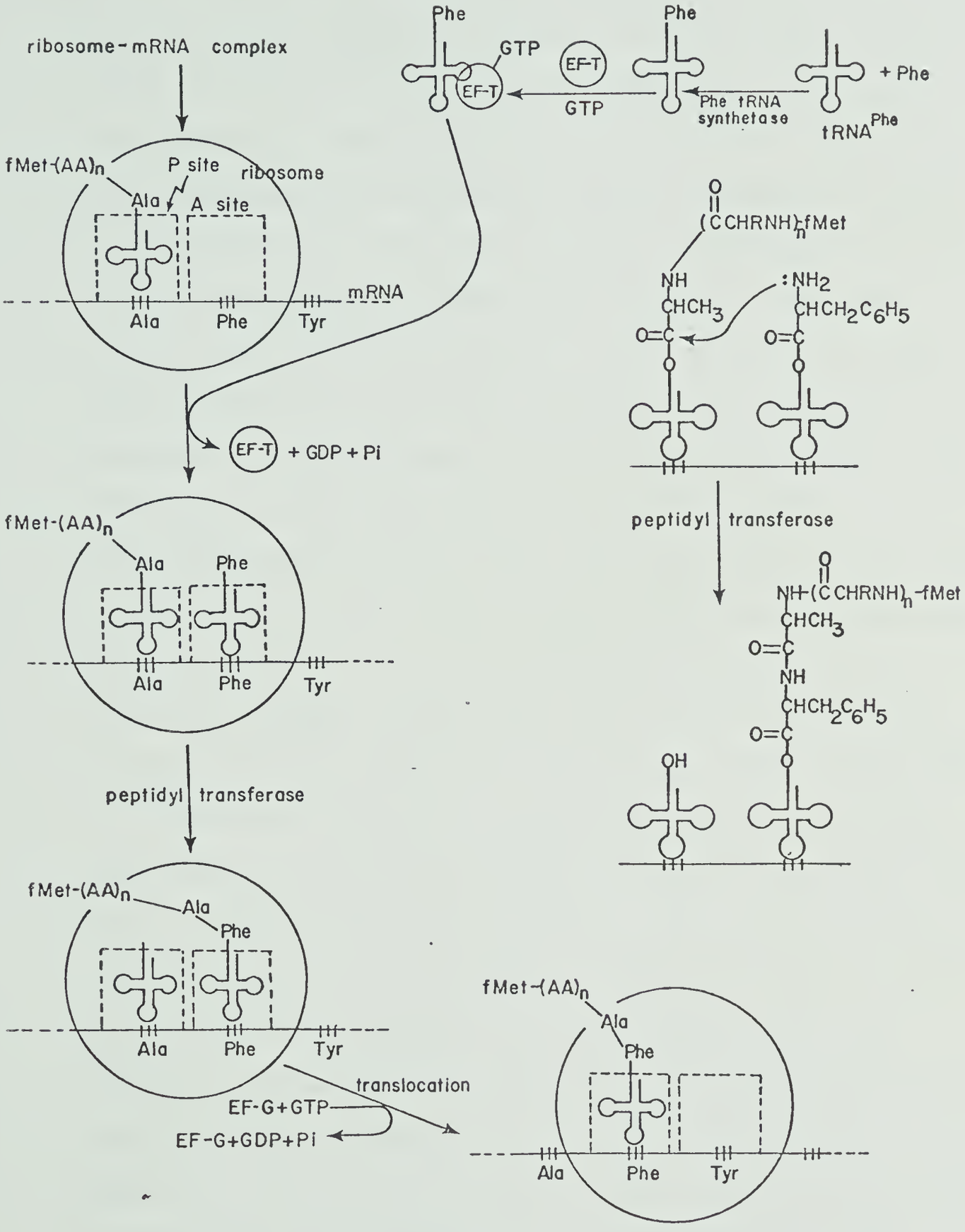
that borohydride reduction of the dialdehyde in an acidic medium resulted in selective reduction of the aldehyde further from the glycosyl bond.[33] The internal hemiacetal structure 23 was proposed by Guthrie to account for this



Scheme IV

selectivity.[39]

Studies on protein synthesis. Oxidized-reduced nucleosides have been used in studies designed to investigate the details of protein synthesis. Scheme V is a simplified outline of the major steps involved in the addition of one amino acid unit to a polypeptide chain. A transfer RNA (tRNA), specific for one amino acid, is acylated by the amino acid at a hydroxyl group of the adenosine residue at

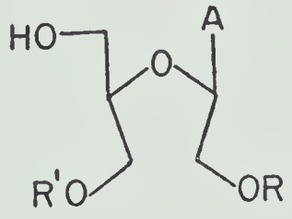

ribosome-mRNA complex
Phe
GTP
EF-T
EF-T
GTP
Phe
Phe tRNA synthetase
+ Phe
tRNA^Phe
fMet-(AA)n
P site
ribosome
Ala
A site
mRNA
Ala
Phe
Tyr
EF-T + GDP + Pi
fMet-(AA)n
Ala
Phe
Ala
Phe
Tyr
peptidyl transferase
fMet-(AA)n
Ala
Phe
Ala
Phe
Tyr
translocation
EF-G+GTP
EF-G+GDP+Pi
fMet-(AA)n
Ala
Phe
Ala
Phe
Tyr
peptidyl transferase

Scheme V

the 3'terminus. This reaction is catalysed by a specific synthetase. The tRNA may be aminoacylated specifically at the 2' or 3' hydroxyl but once this has happened the amino acid migrates rapidly back and forth between the two hydroxyls. After formation of a ternary complex with GTP (guanosine-5'-triphosphate) and an elongation factor Tu this aminoacyl tRNA binds to the A (acceptor) site of the ribosome-messenger RNA (mRNA) complex. The free amino group then forms a peptide bond by attacking the ester linkage through which the polypeptide chain is attached to a tRNA bound to the ribosome P (peptide) site. This reaction is catalysed by peptidyl transferase. In the translocation reaction the tRNA remaining at the P site is released and the entire complex translocates along the mRNA so that the tRNA, now carrying a polypeptide one amino acid longer, shifts from the A site to the P site and the A site is available to the next aminoacyl tRNA.[40]

The first studies on protein synthesis using sugar ring-cleaved nucleosides involved oxidation-reduction of the 3' terminal adenosine of intact tRNA. The modified terminal adenosine of $tRNA^{Phe}_{ox\text{-}red}$ of yeast[41] and *E. coli*[42] was acylated specifically with phenylalanine but the resulting aminoacyl tRNA would not form a complex with Tu and GTP so that binding to the A site was prevented. In addition, non-enzymic binding to the P site was inhibited[42] but not prevented entirely so that the modified tRNA

blocked normal protein synthesis.[43] It was suggested[42] that aminoacylation might take place specifically on the 2' hydroxyl but that transfer to the 3' hydroxyl might be necessary for binding to the A site and subsequent acceptor activity. This transfer occurs rapidly with an intact sugar ring but should be difficult with the ring-opened structure. This proposal received support from the work of Chladek who used aminoacylated oxidized-reduced nucleosides in one of a series of papers concerning the substrate specificity of peptidyl transferase.[44] It had been shown that aminoacylated single nucleosides would act as protein chain acceptors in the peptidyl transferase reaction.[45] (They also prevented amino acid incorporation into proteins, i.e. leucyl adenosine blocked poly U mediated poly Phe synthesis in an unspecified manner.[46]) Chladek showed that compounds 30 and 31 but not 32 were active as acceptors of N-Ac-Phe from N-Ac-Phe-tRNA on *E. coli* ribosomes although the rates with these



30 R = H, R' = Phe

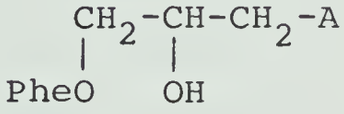
31 R = Phe, R' = Phe

32 R = Phe, R' = H

analogues were slower than with corresponding compounds having an intact sugar ring. However, it was later found that while some *E. coli* tRNAs are initially aminoacylated at only the 2' hydroxyl (including $tRNA^{Phe}$) some are

initially charged at only the 3' hydroxyl and some are initially charged at either position.[47] It was also shown that terminal 2'-aminoacyl-3'-deoxyadenosine acted as a slow acceptor.[43]

It was shown by two different groups that the 3'-aminoacyladenosine analogue 33 would inhibit protein synthesis.[48,49] One group reported it to act as an

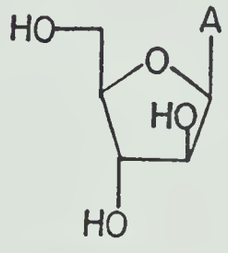


33

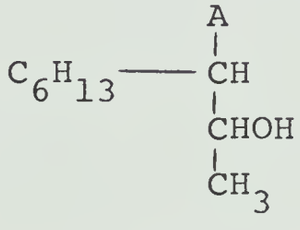
aminoacyl acceptor and inhibit protein synthesis by remaining bound to the ribosome, whereas the other found no acceptor activity and concluded that it inhibited the binding of acceptor substrates to peptidyl transferase.[49]

Studies on adenosine deaminase. The arabino-configuration analogue of adenosine (araA, 34) is a well known nucleoside antibiotic with antiviral and antitumor activity.[50-53] A major problem encountered in its use is its rapid conversion to araH by the enzyme adenosine deaminase. This prompted investigation of the substrate specificity of adenosine deaminase in order to find similar drugs which are poor substrates of this enzyme or to find inhibitors of adenosine deaminase which could be co-administered with araA. To this end Rossi and Lerner synthesized

and evaluated a number of ring-opened adenosine analogues, with modifications to both sugar[54] and base,[55] as enzyme substrates or inhibitors. Schaeffer and coworkers synthesized and evaluated a large number of 9-hydroxyalkyl adenines and 6-substituted 9-hydroxyalkyl adenines with alkyl chains of up to nine carbons.[56-60] A number of these compounds were found to be good inhibitors, especially *erythro*-9-(2-hydroxy-3-nonyl)adenine (EHNA, 35) which was found to be an effective inhibitor of adenosine deaminase activity also in cultured cells.[61]



34



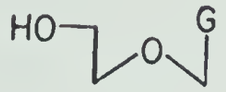
35

Open chain nucleosides-use as drugs. An important reason for synthesizing the compounds made in the present study was their potential as antimetabolites. There are a number of nucleosides in clinical use and so analogues are constantly being synthesized and tested for biological activity. AraA (34) was mentioned above as a widely studied drug, 2'-amino-2'-deoxy-guanosine was recently isolated as an antibiotic,[62] and a number of 6-substituted purine nucleosides have shown antimetabolic activity.[63] The syntheses of many purine nucleoside analogues

substituted at the 2', 3', and 6 positions have been published in the last few years including 2'-azido- and 2'-amino-2'deoxyadenosine,[64,65] 9-(2-azido-2-deoxy-β-D-arabinofuranosyl)adenine, 9-(2-amino-2-deoxy-β-D-arabino-furanosyl)adenine,[66] and 2'-halo-2'deoxyadenosines.[67] Some years ago Jahn made a series of 5'-substituted adenosines.[68] Preliminary results have indicated that 2'-azido-2'-deoxyaraA has cytotoxic and antiviral properties comparable to those of araA.[66] 2'-Amino-2'-deoxyadenosine and 2'-amino-2'-deoxy araA also show some activity.[66,69] The compounds synthesized in the present study are simple ring-opened analogues of these compounds with additional flexibility allowing them to assume conformations similar to the ribo-, arabino-, xylo-, and lyxo- configurations of the parent intact-ring compounds.

Some open-chain analogues synthesized to date have shown interesting biological activity. 9-Alkyl derivatives of 6-chloropurine and 6-mercaptopurine were found to be inhibitors of adenocarcinoma 755[70] and this led to the synthesis of a number of similar analogues.[71] Corey and George found that ATP_{ox} (the dialdehyde of ATP) is an effective inhibitor of CDP and ADP reduction by ribonucleoside reductase of Ehrlich tumor cells,[72] and that AMP_{ox} and A_{ox} inhibit both RNA and DNA synthesis in Ehrlich tumor cells in culture, with A_{ox} being effective *in vivo* as well.[73] Schaeffer and coworkers recently

reported that compound 36 was ten times as potent as the

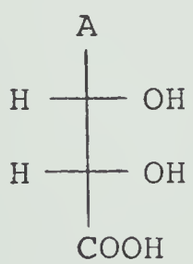


36

clinically employed 5-iodo-2'-deoxyuridine against herpes virus infections and also had low host toxicity.[74] The corresponding adenine compound showed very weak activity. Wolfrom, Horton, and coworkers have published a series of papers[75] describing the synthesis of open-chain nucleoside analogues. They used bases with known anti-tumor potential, such as 6-mercaptopurine, and many common five and six carbon sugars, often substituted with an SEt group at the 1' position. They made these compounds for evaluation as antimetabolites but have not published any biological data. They reasoned that the straight-chain sugar structure of these compounds might facilitate their transport across cell membranes *in vivo*[75g] and in this regard the work of Holy[76] is important. He synthesized a large number of hydroxypropyl, dihydroxy-propyl, and dihydroxybutyl derivatives of purines and pyrimidines, having both the S (naturally occurring) and R configurations. None of the compounds inhibited the growth of *E. coli* cultures. It was found that the ribo-side of 2-pyrimidinone has high bacteriostatic activity whereas its 2,3-dihydroxypropyl derivative is completely inactive. Holy pointed out that Showdomycin antagonists

in an *E. coli* system, which appear to act by competing with Showdomycin for binding sites on the cell surface prior to transport across the cell membrane, can have various structural modifications in the base moeity but the structural requirements for the sugar are very strict.[77] It had also been demonstrated that the sugar structural requirements for the cytidine transport system of *Bacillus subtilis* are fairly severe, with adenosine having a high affinity for the system but 9-(2,3-dihydroxypropyl)adenine having none.[78] Therefore, Holy concluded that the lack of activity observed with his compounds against *E. coli* could quite possibly be due to their failure to be transported across the bacterial cell walls. As well, Boos *et al.*[79] found that $ATP_{ox\text{-}red}$ showed carrier-linked binding to rat liver mitochondria and acted as a competitive inhibitor of ATP uptake but was not itself transported across the mitochondrial membrane.

The Japanese pharmaceutical industry has shown some interest in the hypocholesterolemic substance eritadenine (37), an open chain nucleoside isolated from a species

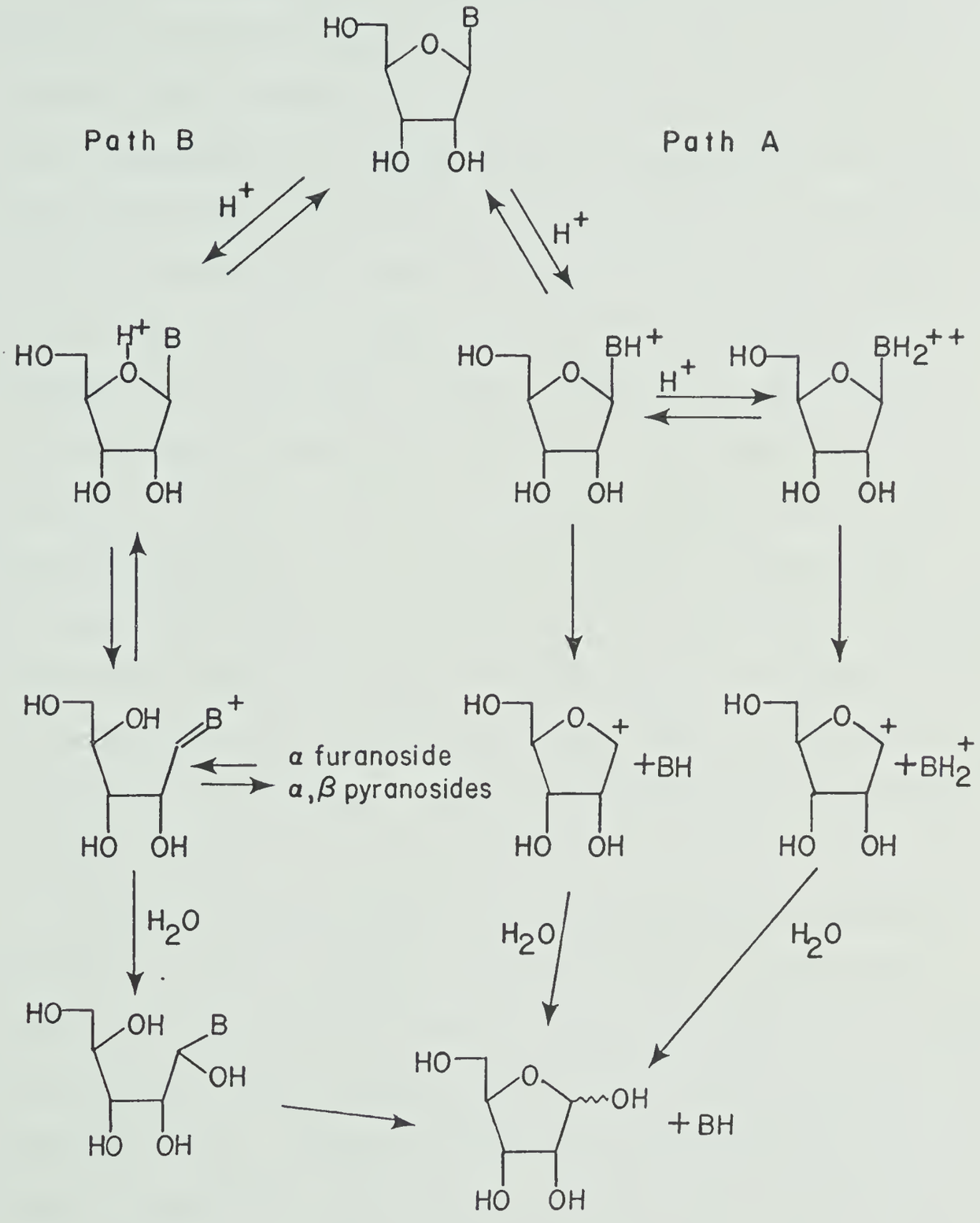


37

of mushroom.[80,81] Many analogues have been synthesized[82] but there have been no reports of the biological behavior of these compounds.

ACID CATALYSED HYDROLYSIS OF NUCLEOSIDES.

Nucleic acids were first hydrolysed in acid in 1891 by Kossel[6] and this led to the identification of the heterocyclic bases, but it was not until 1957 that a mechanism was proposed for acid catalysed hydrolysis of the glycosyl bond. Kenner[83] proposed the mechanism labelled path B in Scheme VI. First the sugar ring oxygen is protonated followed by a ring opening with C1'-O bond cleavage and formation of a Schiff's base. The Schiff's base is attacked by water with subsequent breakdown to products. The controlling factor would probably be the ability of the glycosidic bond nitrogen to sustain a positive charge. Kenner acknowledged that protonation should occur first on the base but reasoned that hydrolysis could occur as long as the proton could be transferred easily from the base to the sugar or if the positive charge was delocalized enough to allow a second protonation. Dekker supported this mechanism and emphasized the importance of the ease of transferring a proton from base to ring oxygen in determining the rate of hydrolysis.[84] This Schiff base mechanism was based largely on analogy to the acid catalysed hydrolysis of glycosylamines. Some


Path B
Path A
α furanoside
α,β pyranosides
+BH
+BH2
H2O
H2O
H2O
+BH

Scheme VI

experimental data consistent with this mechanism was observed.[85]

This mechanism was first seriously challenged by Shapiro and Kang[86] on several grounds. Their kinetic data was more compatible with an Al mechanism, shown as path A in Scheme VI, and in addition they re-interpreted data from other authors[85] in support of this Al mechanism. In the Al mechanism, the base is protonated in a rapid equilibrium step followed by rate-limiting unimolecular glycosyl bond cleavage. This liberates the free base and a cyclic oxy-carbonium ion which adds water to give D-ribose. Another piece of evidence against the Schiff base mechanism was the failure to detect α furanoside or α or β pyranoside byproducts during nucleoside hydrolyses. If a Schiff base intermediate were formed it should do so reversibly with reclosing of the ring to give the α anomer and pyranosides. Such byproducts were observed in glycosyl amine hydrolyses.[87]

Further evidence for an Al mechanism was provided by the pH rate profiles. Zoltewicz[88,89] reported that plots of log k (where k is the observed pseudo-first order rate constant) vs. pH were linear even in the region where pH $\tilde{=}$ pK_a. Others noted small curvatures[90] or a small plateau[91] in this region, but in all cases the plots were linear with the same slope when pH < pK_a and did not show the maxima at low pH characteristic of

glycosyl amine hydrolyses.[92] When pH $\leq$ pK_a both mono- and diprotonated species are involved. The diprotonated base is produced less easily since the monoprotonated base is already positively charged. However, if the Al mechanism is operative the diprotonated base species should hydrolyse much faster and so a combined linear rate profile is possible. If the Schiff base mechanism were correct, not only would the diprotonated base form less readily but it would also disfavor Schiff base formation so that the rate would decrease as the pH was lowered. Zoltewicz reported[89] that according to his kinetic data in 1M perchloric acid at 30°C, diprotonated species make up about 10% of the total. However, hydrolysis of the monocation was negligible under those conditions which meant that bond cleavage in the dications must be at least 10^3 times faster.

Zoltewicz found that the 1,7-dimethylguanosine iodide salt hydrolyses much faster in dilute acid than guanosine despite having a positively charged base and no proton to transfer to the sugar.[88] This is consistent with an Al mechanism but not with the Schiff base route. Several studies have indicated that the hydrolysis of nucleosides is subject to specific acid catalysis,[89,90,93] which is consistent with an Al mechanism. In glycosyl amine hydrolyses, which proceed via a Schiff base intermediate, general acid catalysis by acetic acid/acetate

buffers has been observed.[94] In only one instance of nucleoside hydrolysis have the byproducts expected from a Schiff base intermediate been detected; Cadet and Theoule[95] found that in the hydrolyses of thymidine and 2'-deoxyuridine up to 10% of starting material was converted to α furanoside, β pyranoside, or α pyranoside. They were unable to detect these side products in the hydrolysis of 5-bromo-2'deoxyuridine or 2'-deoxycytidine.

To establish whether the glycosyl bond cleavage was unimolecular (A1) or bimolecular (A2 - involving backside nucleophilic attack by water with simultaneous glycosyl bond cleavage) Townsend and coworkers[96] studied the hydrolysis rates of purine N-7 ribofuranosides and compared them to N-9 ribofuranosides. The N-7 isomers must exist in the *anti* conformation since the 6-substituent is oriented towards the sugar. In this orientation it should sterically hinder the reaction centre and decrease the rate by increased steric compression in the transition state if the mechanism were A2. If the mechanism were A1 the transition state would be less crowded than the ground state and the rate would be accelerated. They found that the 7-isomers hydrolyse faster in all the purines studied except guanosine, and they rationalized this result in terms of different protonation sites on the base. A theoretical study also concluded that the A1 mechanism was valid and that water could not participate

in a bimolecular cleavage step since steric repulsions prevent its approach from the angle that would assist departure of the base.[97]

Very recently Romero *et al.*[98] determined the α deuterium (secondary) kinetic isotope effects in the hydrolyses of adenosine and inosine. Their k_H/k_D values were all close to 1.20 which indicates considerable carbonium ion character in the transition state. The C-N bond must be completely or almost completely cleaved in the transition state. The simplest explanation of these results is the A1 mechanism. Romero *et al.* point out that their values do not completely rule out a transition state in which C-N bond cleavage is quite advanced but there is some nucleophilic participation by water (i.e. A2 character). They also considered the possibility of complete dissociation in the transition state with diffusion separation of the carbonium ion and free base (or base protonated cation) being the rate limiting step. Young and Jencks[99] estimated the lifetimes of the carbonium ions derived from glycopyranosides to be extremely short (10^{-11} to 10^{-15} seconds) so that such ions cannot be reaction intermediates. Some kind of stabilization such as nucleophilic participation by water would be involved in the formation of these carbonium ions. On the other hand, Jones *et al.*[100] have speculated that the carbonium ions derived from glycopyranosides are approximately as

stable as the diphenylmethyl carbonium ion, with lifetimes in the order of 10^{-9} seconds. Their k_H/k_D value for the hydrolysis of 3-chloro-1-(β-D-galactopyranosyl)pyridinium ion was similar to those of Romero *et al.* and best explained by unimolecular cleavage.

The value of $\Delta S^{\ddagger}$, the entropy of activation, should readily distinguish between a unimolecular ($\Delta S^{\ddagger}$ would be positive) or bimolecular ($\Delta S^{\ddagger}$ would be negative) mechanism. However, reported values of $\Delta S^{\ddagger}$ vary widely. $\Delta S^{\ddagger}$ values for hydrolysis of adenosine in HCl range from +6.1 eu to -10.8 eu. Helvesi *et al.*, who reported the $\Delta S^{\ddagger}$ value of -10.8 eu,[90] stated that their kinetic data generally agrees with the Al mechanism and rationalized this value by invoking neighboring group participation by the 2' hydroxyl group. Such participation would create a more ordered transition state. They also invoked this neighboring group participation to explain why their values of $\Delta H^{\ddagger}$ (enthalpy of activation) for 2'-deoxyadenosine and 2'-deoxyguanosine are higher than those for adenosine and guanosine. Their values of $\Delta G^{\ddagger}$ (Gibbs free energy of activation) are lower for the deoxy compounds, in agreement with the faster hydrolysis rates observed for 2'-deoxynucleosides, due to much higher values of $\Delta S^{\ddagger}$ (+8.4 eu for 2'-deoxyadenosine).

The exact role of the 2' hydroxyl group in nucleoside hydrolyses is not clear. Garrett suggested[93] that it may

compete as a proton acceptor site as well as exerting an inductive withdrawing effect. Spectral data indicate that hydrogen bonding can occur between the 2' hydroxyl and N3 of adenine,[101] which would lower the inductive effect of the hydroxyl group. Garrett found that the hydrolysis of 2'-C-methyl adenosine is slower than that of adenosine although on strictly inductive grounds it should be at least as fast.[93] This could be due to steric hindrance of solvation of the carbonium ion developing at C1' or to interference with the 2'OH-N3 interaction so that the inductive effect of the 2'OH is not lowered by H-bonding. 2'-O-Methyladenosine is also more stable than adenosine in aqueous acid, possibly because it cannot hydrogen bond to the base.[102] This hydrogen bond has been observed only in organic solvents. Its existence in aqueous media is supported by some indirect experimental evidence but is not firmly established. Therefore, interpretations of the above results in terms of H-bonding effects, involving the neutral base are probably invalid since the data were obtained in aqueous acid at temperatures of 41°C[102] and 80°C.[93]

Whatever the overall role of the 2' hydroxyl group involves, it is generally found that acid stability of nucleosides is proportional to the number of hydroxyl groups on the sugar ring.[93,103] Other electron withdrawing substituents on the sugar also increase the stability.

This, of course, is consistent with an A1 mechanism since withdrawing groups destabilize the developing carbonium ion character at C-1'. These effects are decidedly strongest at the 2' position. This has led to the use of withdrawing groups such as substituted benzene-sulfonates to stabilize nucleoside derivatives while removing acid-labile protecting groups under conditions that would normally result in glycosyl bond cleavage.[103]

The nature of the base moiety has a controlling effect on the rate of hydrolysis. The order of ease of hydrolysis of the common naturally occurring bases is G > A > C > U (T).[104] It is probable that different nucleosides hydrolyse by different mechanisms or by a combination of concurrent mechanisms. 2'-Deoxyuridine and thymidine gave byproducts characteristic of a Schiff base mechanism as described above.[95] In another study of pyrimidine nucleoside hydrolysis, substitution on the base with either electron withdrawing or electron donating substituents increased the rate of hydrolysis.[105] However, for purine nucleosides, (which are the subject of the present study) the A1 mechanism is fairly well established and appears to be in operation uniformly.

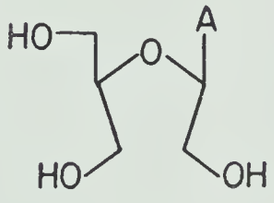
The position of the glycosyl bond is important, since 7-ribosyl purines have faster hydrolysis rates than 9-ribosyl purines,[96] and 3(β-D-ribofuranosyl)adenine has a very fast rate.[106] α and β anomers hydrolyze with

similar rates.[107] Base orientation was suggested to be important in a compound locked in the *syn* conformation by a bulky C8 substituent,[91] but steric factors could also be involved. Base substitution follows a regular pattern, at least in adenine nucleosides. Electron withdrawing substituents should increase the leaving ability of the base but decrease its ease of protonation. Donating groups should have the opposite effects. Effects on the leaving ability of the base should be greatest from substituents on the imidazole ring (close to N9). Effects on the ease of protonation should also be greatest involving substituents in that region since formation of the dication, the species said to be almost exclusively the reacting species at low pH,[89] probably occurs by protonation at N7.[93] In some instances the opposing effects may cancel to a large extent. Rate data on hydrolysis of base-substituted adenine nucleosides show that withdrawing substituents accelerate the rate while donating substituents retard it,[93,96] in accordance with glycosyl bond cleavage being the rate determining step.

DISCUSSION

A NOTE ON NOMENCLATURE.

The oxidized-reduced nucleosides are named formally in Table I. There are alternative ways of naming them. For example, Lerner[38] named oxidized-reduced adenosine (25) 1-0-(1,3-dihyroxy-2-propyl)-1-(adenin-1-yl)-1(R), 2-ethanediol. For the sake of clarity, in this thesis these opened-chain compounds will be named informally as substituted oxidized-reduced adenosines. The carbons retain the numbers assigned to them in the parent intact-ring nucleosides. For example, compound 49 is 2'-chloro-2'-deoxyadenosine$_{ox\text{-}red}$ and compound 61 is 5'-0-tosyl-adenosine$_{ox\text{-}red}$.



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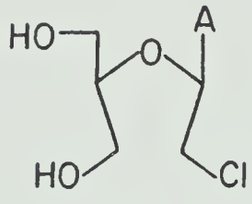

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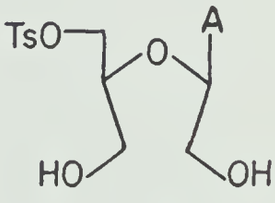


61

SYNTHESIS OF OPENED-CHAIN ANALOGUES.

The dialdehyde of adenosine (14) was prepared by periodate oxidation according to literature procedures and its properties were found to be similar to those described by Hansske *et al.*[35,37] and Jones *et al.*[36] A concentrated aqueous suspension of adenosine (10 mmole nucleoside in

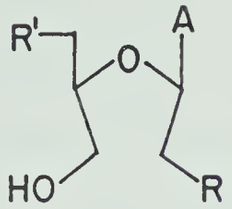


TABLE I

Compound	R=	R'=	Formal Name
25	OH	OH	2(R)-(9-adeninyl)-4-hydroxymethyl-3-oxa-pentan-1,5-diol
43	OTs	OH	2(R)-(9-adeninyl)-4-hydroxymethyl-1-O-(p-toluenesulfonyl)-3-oxapentan-1,5-diol
48	N_3	OH	4(R)-(9-adeninyl)-5-azido-2-hydroxymethyl-3-oxapentan-1-ol
49	Cl	OH	4(R)-(9-adeninyl)-5-chloro-2-hydroxymethyl-3-oxapentan-1-ol
50	Br	OH	4(R)-(9-adeninyl)-5-bromo-2-hydroxymethyl-3-oxapentan-1-ol
51	I	OH	4(R)-(9-adeninyl)-2-hydroxymethyl-5-iodo-3-oxapentan-1-ol
53	H	OH	4(R)-(9-adeninyl)-2-hydroxymethyl-3-oxapentan-1-ol
55	NH_2	OH	4(R)-(9-adeninyl)-5-amino-2-hydroxymethyl-3-oxapentan-1-ol
56	NHPhe	OH	4(R)-(9-adeninyl)-5-[N-(L-phenylalanyl)]amino-2-hydroxymethyl-3-oxapentan-1-ol
57	NHLeu	OH	4(R)-(9-adeninyl)-5-[N-(L-leucyl)]amino-2-hydroxymethyl-3-oxapentan-1-ol

(continued...)

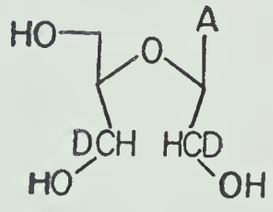
TABLE I (continued)

61	OH	OTs	2(R)-(9-adeninyl)-4(S)-hydroxymethyl-5-0-(p-toluenesulfonyl)-3-oxapentan-1,5-diol
63	OH	N_3	2(R)-(9-adeninyl)-4(R)-azidomethyl-3-oxapentan-1,5-diol
65	OH	Cl	2(R)-(9-adeninyl)-4(S)-chloromethyl-3-oxapentan-1,5-diol
67	OH	NH_2	2(R)-(9-adeninyl)-4(R)-aminomethyl-3-oxapentan-1,5-diol
69	OH	H	2(R)-(9-adeninyl)-4(R)-methyl-3-oxapentan-1,5-diol

40 ml water) becomes clear rapidly upon addition of sodium periodate, but a polymeric precipitate (structure 24 in Scheme III) begins to form within an hour. The proton NMR spectrum of this precipitate shows no peaks in the aldehyde proton region and all peaks are broad. Its UV spectrum in water is similar to that of adenosine (λ_{max} = 258 nm, ε = 14,000). The product in the supernatant solution migrates as one spot, Rf = 0.25, when analysed by tlc on silica gel with 10% methanol/chloroform. It streaks badly in some other solvent systems such as ethyl acetate/n-propanol/water (4:1:2, upper phase), presumably due to the different hydrated structures involved as indicated in Scheme III. Allowing the oxidation reaction mixture to stand in the refrigerator for 1-2 days provided a simple method for separating the nucleoside from the water soluble iodate salt. Yields of up to 87% of the polymeric dialdehyde were obtained in this way. This polymeric material is readily reduced by sodium borohydride. Analogous oxidations of concentrated solutions or suspensions of 5'-substituted adenosines also gave polymeric precipitates. They usually precipitated in low yields over extended time periods and so the reduction of these dialdehydes was carried out directly *in situ* by addition of borohydride to the oxidation reaction mixture. In more dilute solutions the polymeric adenosine dialdehyde forms slowly if at all. The oxidation is complete within

one hour at room temperature and addition of borohydride to this solution gives the oxidized-reduced product 25 within another hour. Salts were removed at this stage by adsorption of the nucleoside onto charcoal or by ion exchange chromatography.

Reduction with sodium borodeuteride gives the analogue 38 with deuterium labels substituted at the 2' and 3' carbons. Although the 3' and 5' hydroxyl groups of adenosine$_{ox\text{-}red}$ (25) are chemically identical they are prochirally distinct in the proton NMR spectrum in DMSO, appearing as sharp triplets at δ 4.43 and δ 4.68. The spectrum of 38 allows these peaks to be assigned since the 3' hydroxyl signal (at δ 4.68) now appears as a doublet.



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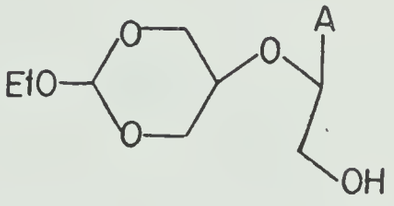
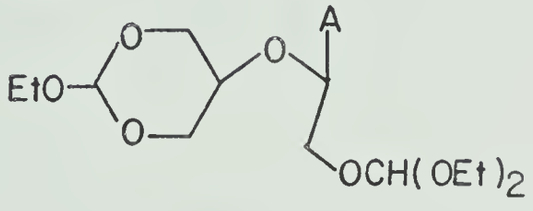


39

The strategy for preparing the 2'-substituted derivatives was to block the 3' and 5' hydroxyls of the trialcohol 25 with a cyclic blocking group and then perform transformations on the free 2' position. Cyclic blocked structures involving the 2' hydroxyl should not form since they involve an eight-membered ring. Cyclic

blocking groups are commonly used on carbohydrates and a number of preparations are described in the literature. (For example, see references 108 and 109, and references therein.) The desired blocked compounds were surprisingly difficult to make. Attempted preparations of the isopropylidene, benzylidene, and methylidene derivatives of 25 by literature procedures[109] gave only starting material. However, preparation of the ethoxymethylidene derivative 39 by the procedure of McCarthy *et al.*[110] proceeded smoothly in good yield. The reaction always gave rise to one side product, presumably the completely blocked derivative 40, which could not be completely eliminated by reducing the molar ratio of triethylorthoformate and could not be hydrolysed to 39 without concomitant hydrolysis of the cyclic blocking group.



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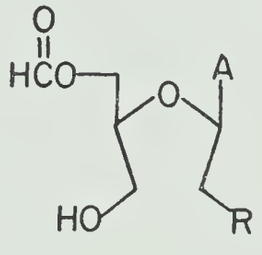
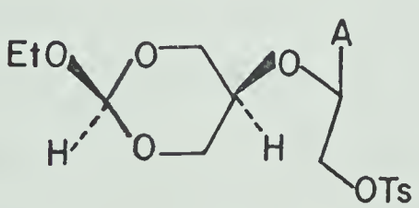


41

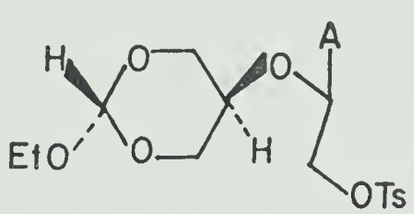
The ethoxymethylidene group is very acid labile. Deblocking could be carried out by treatment of 39 with 80% acetic acid for five minutes or with 99% trifluoroacetic acid for one minute at room temperature. Stirring overnight in water results in almost complete deblocking.

The procedure used to deblock the 2'-substituted 3',5'-ethoxymethylidene compounds (42, 44-47) involved refluxing in ethanol with a trace of acetic acid for a few hours. A small amount of partially deblocked compound (41) was produced but this was easily removed by chromatography on silica gel or else by hydrolysis to the desired free compound in methanolic ammonia (pH 9). Deprotection reactions were monitored for adenine (resulting from possible glycosyl bond hydrolysis) but its presence was never observed. Appreciable deblocking of 39 occurred during preparative tlc on silica gel plates. Therefore, triethylamine was added to the eluting solvent when 39 was separated from 40 on a silica gel column.

The blocked compound 39 was converted to its 2'-O-tosyl derivative (42) using tosyl chloride in pyridine. The product is an almost equal mixture of *cis* and *trans* isomers. These isomers migrate as one spot during tlc on



cis-42

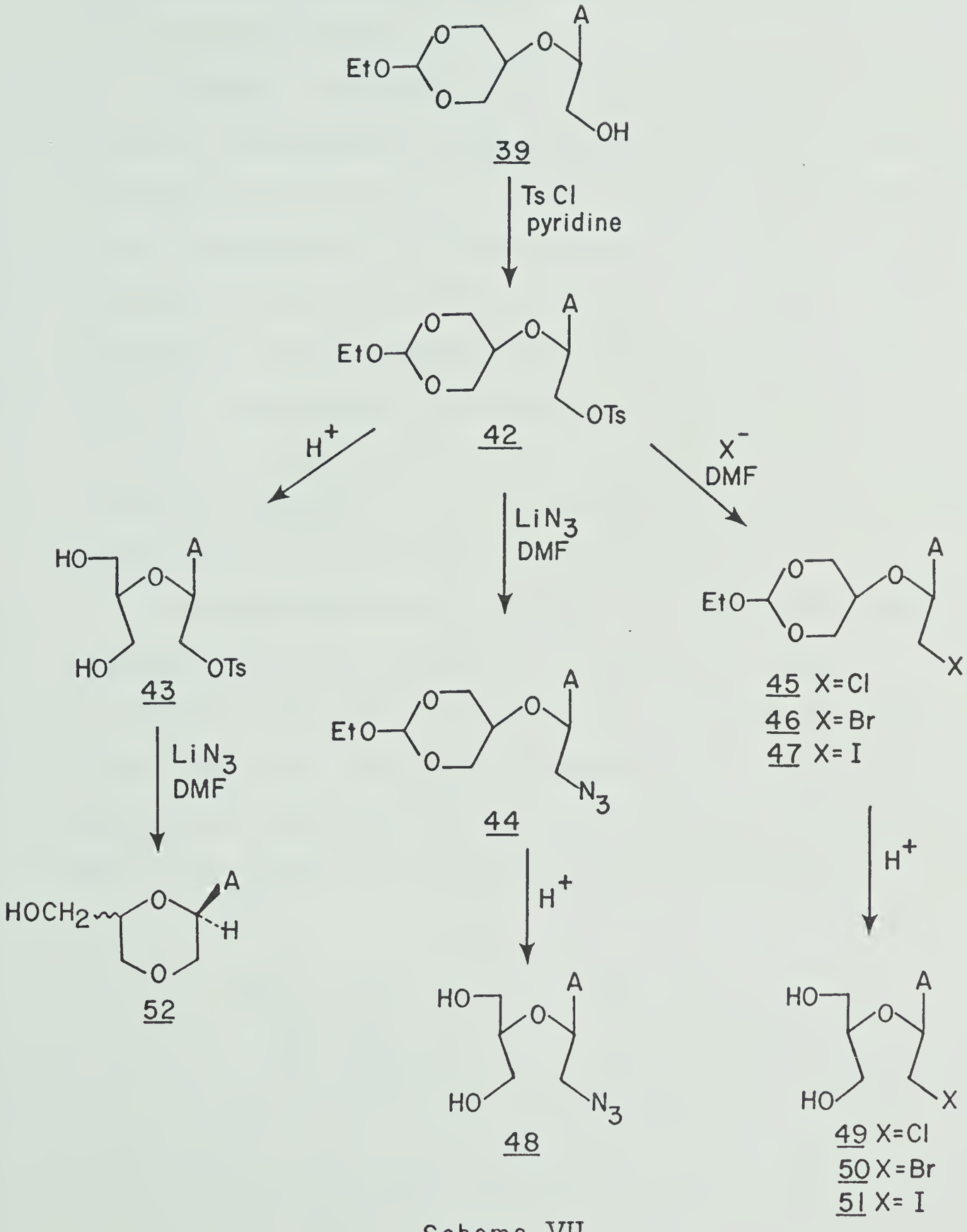


trans-42

silica with 10% methanol/chloroform a solvent. When 5% methanol/chloroform was used the presence of two isomers

was apparent. Four crops of 42 were obtained from ethanol by diffusion with pentane, and successive crops had an increasing proportion of the faster running isomer. The diastereomeric orthoester protons appear as singlets at δ 5.22 and δ 5.29 in the proton NMR spectrum in DMSO. The successive crystallization crops have more of the isomer with the signal at δ 5.29. The proton NMR spectrum of 42 in $CDCl_3$ also exhibited two signals for the more upfield of the two adenine ring protons, which is normally H2. The relative peak heights of these two base singlets corresponded in each crystallization crop to the relative peak heights of the two orthoester proton singlets. This *cis, trans* isomerism was also observed during tlc of the corresponding 2'-azido- (44), 2'-chloro- (45), 2'-bromo- (46), and 2'-iodo- (47) compounds.

Deblocking of 42 under mild acid conditions gave 2'-O-tosyladenosine$_{ox\text{-}red}$ (43). The 2'-azido- and 2'-halogen substituted oxidized-reduced adenosines (48-51) were prepared by nucleophilic substitution of the tosyl group of 42 followed by deblocking in acid as illustrated in Scheme VII. Presumably the halogen substituted compounds could also have been prepared by substitution reactions with the unblocked 2'-O-tosyladenosine$_{ox\text{-}red}$ (43). However, the blocked derivatives are soluble in organic solvents and extraction into methylene chloride was a convenient method of separating them from the salts

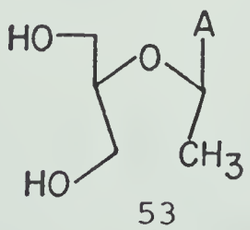

EtO
A
OH
39
Ts Cl
pyridine
OTs
42
H+
X-
DMF
LiN3
DMF
HO
OTs
43
45 X=Cl
46 X=Br
47 X=I
N3
44
HOCH2
H
52
48
49 X=Cl
50 X=Br
51 X=I

Scheme VII

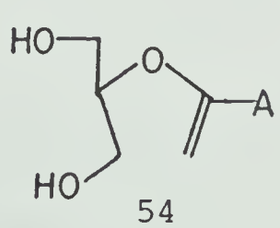
in the reaction mixture. Azide anion is a strong base and the reaction of 43 with lithium azide under the normal substitution conditions gave the dioxane derivative 52 as the only product.

A number of methods for converting primary alcohols directly to halides have been described in the literature.[111] Attempts to prepare the 2'-chloro compound 45 directly from the primary alcohol 39 using thionyl chloride gave complex mixtures of products. The procedure of Verheyden and Moffatt[111h] using triphenylphosphine and carbon tetrachloride resulted in isolation of starting material. Similarly, treatment of the alcohol 39 with triphenoxymethyl phosphonium iodide[111c] as a potential route to the 2'-iodo derivative 47 yielded only starting material.

2'-Deoxyadenosine$_{ox\text{-}red}$ (53) was prepared by catalytic hydrogenolysis of 2'-bromo-2'-deoxyadenosine (50). Potassium hydroxide was added to the reduction mixture since the deoxy- analogue is susceptible to glycosyl bond hydrolysis catalysed by the HBr produced in the reaction. The 1'-2' unsaturated analogue 54 was prepared by



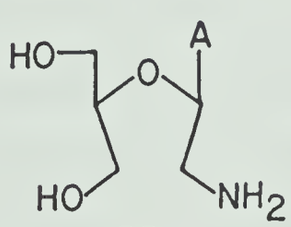
53



54

chromatography of 2'-iodo-2'-deoxyadenosine$_{ox\text{-}red}$ (51) on a basic anion exchange resin (Dowex 1-X2 hydroxide form) or by stirring 51 with the same resin in methanol/water for two hours. The dioxane derivative 52 was not observed as a byproduct of this reaction. Compound 54 is somewhat unstable and is difficult to isolate and crystallize in a pure form.

Preparation of 2'-amino-2'-deoxyadenosine$_{ox\text{-}red}$ (55) by reduction of the azido- analogue 48 over palladium on carbon appeared to be successful from tlc and mass spectral data, but attempts to isolate and crystallize the product always resulted in gummy material. Preparations of the hydrochloride and acetic acid salts of this material also resulted in gums. However, reduction of 48 with Raney nickel/hydrazine gave 55 as a crystalline analytically pure material. This amino- analogue was



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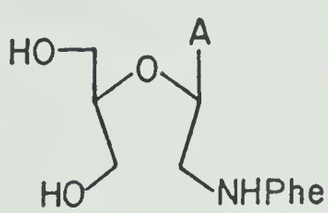

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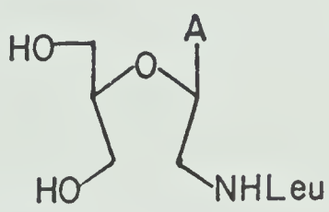


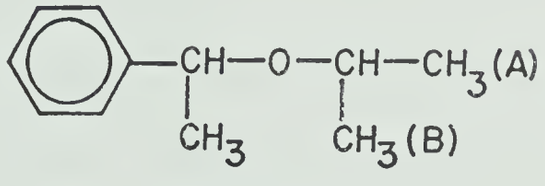
57

coupled with active esters of amino-protected phenylalanine and leucine in a procedure similar to that of Robins *et al.*[112] to give the 2'-aminoacylated derivatives 56 and

57 after deprotection.

An interesting feature of the 2'-substituted oxidized-reduced adenosines is the fact that the chemically equivalent prochiral 3' and 5' hydroxymethyl groups are distinct in the proton NMR spectra in DMSO. Compounds 25, 43, and 48-51 have one hydroxyl signal at δ 4.70-4.79, and the other at δ 4.44-4.50. One methylene signal at δ 3.48-3.53 is coupled to the downfield hydroxyl proton and the other methylene at δ 3.19-3.23 is coupled to the upfield hydroxyl proton. This observed non-equivalence is possibly due to a restricted conformation resulting from hydrogen bonding between the 3' or 5' hydroxyl group and N_3 of adenine. H-bonding between the 5'OH and N3 of purine nucleosides with intact sugar rings has been observed in X-ray crystall structures,[113] and in solution in both organic solvents[114,115] and H-bonding solvents such as ammonia or water.[116] This H-bond has been invoked in other studies to account for high populations of gauche-gauche rotamers about the C4'-C5' bond[117,118] and is thought to be a major factor influencing the relative populations of *syn* and *anti* rotamers about the glycosyl bond.[119-121] H-bonding usually shifts a proton signal downfield. From the proton NMR spectrum of the deuterated compound 38 it was determined that the 3' hydroxyl proton is the more downfield and all of these compounds have similar chemical shift values. It is not apparent why the

3' hydroxyl group should H-bond in preference to the 5' hydroxyl, but the conformational features of nucleosides are hard to predict. It is likely that the non-equivalence is caused by differential shielding by the ring current of the adenine base, particularly since the chemical shift difference between the signals of the methylene groups is large (0.30 ppm at 100 MHz). Shugar and coworkers found that H-bonding between the 5'hydroxyl group and the ionized 2' hydroxyl of arabinosyl pyrimidine nucleosides at high pH caused the signal for the 5' methylene protons to shift by only 0.10 ppm.[122] In a study of long range non-equivalence due to molecular asymmetry, Roberts and coworkers found that the signals for methyl groups (A) and (B) of compound 58 chemical shifts differing by less than 0.1 ppm.[123] These methyl groups are analogous to the 3' and 5' methylene groups of the



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2'-substituted ring-opened nucleosides. However, Robins and coworkers[124] observed much larger anisotropic effects (0.3 ppm and larger) on the chemical shifts of the 2' protons of the cyclic 3',5'-monophosphates of purine 2'deoxy nucleosides. Whatever the exact conformational

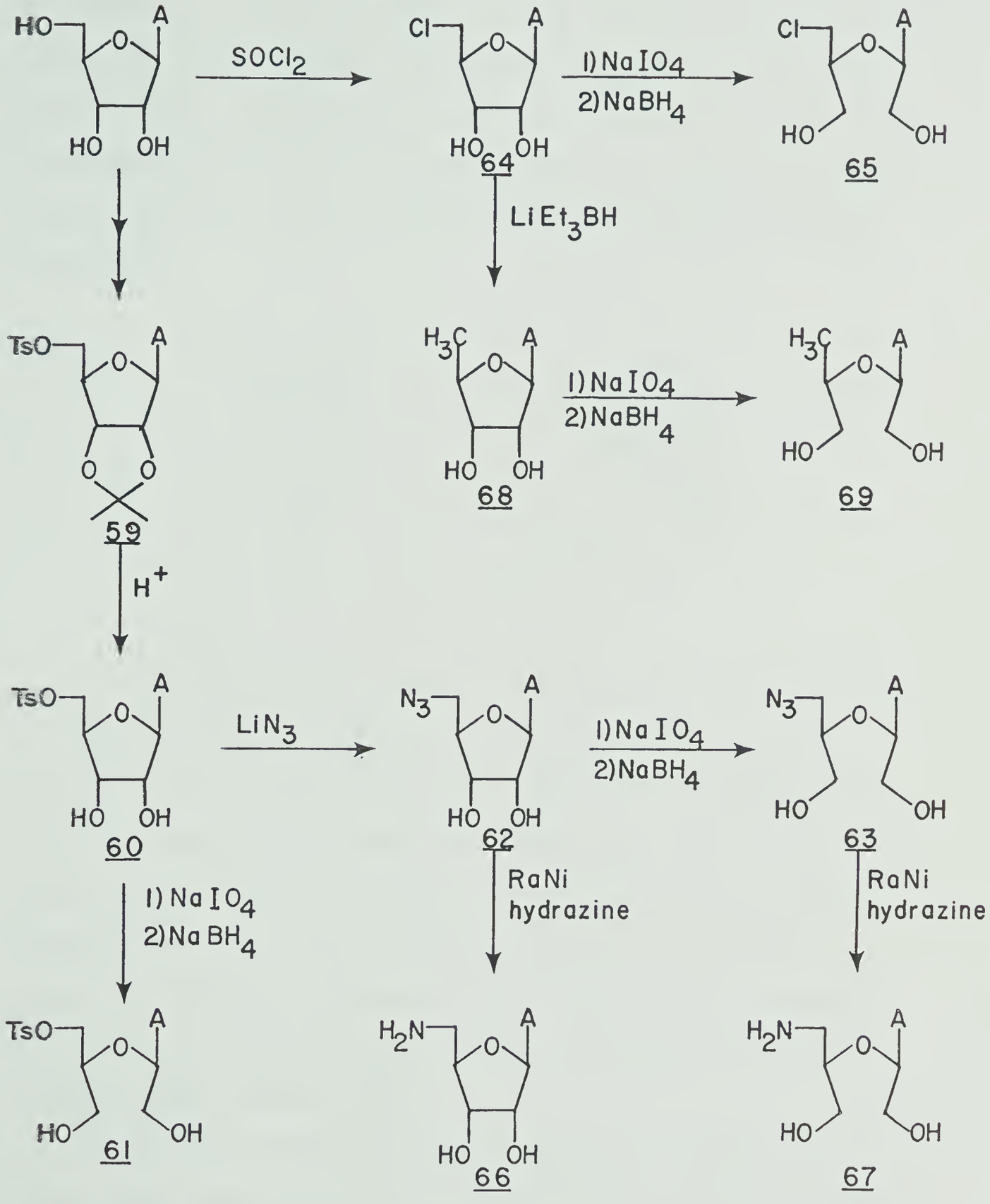
cause of this non-equivalence may be, the spectrum of the 1'-2' unsaturated compound 54 shows the 3' and 5' hydroxymethyl groups equivalent as expected.

The 5'-substituted adenosines prepared in this study have been synthesized previously by Jahn[67] and other workers. 5'-O-Tosyl-2',3'-O-isopropylidene adenosine (59) was prepared by the general procedure of Kuhn and Jahn.[125] The isopropylidene group was removed within 15 minutes at room temperature by treatment with 90% trifluoroacetic acid. The resulting 5'-O-tosyladenosine (60) was converted to its ring-opened analogue (61) by the same periodate oxidation-borohydride reduction procedure as was used to make adenosine$_{ox-red}$. For solubility considerations, this reaction was effected in 3:1 water/DMF. 5'-O-Tosyladenosine$_{ox-red}$ decomposed to a small extent when stored at room temperature, but it was stable for an extended period at -10°C.

When the 5' position of an adenine ribonucleoside is substituted with a good leaving group, cyclonucleoside salt formation can occur via attack on the 5' position by N3 (Scheme II). Under conditions necessary for nucleophilic attack of halogen on 2',3'-isopropylidene-5'-O-tosyladenosine, cyclonucleoside salts would be the only products.[18] Jahn[67] effected such substitutions in acetic anhydride or formic-acetic anhydride as solvents so that the 6-amino group was acylated. This markedly retards

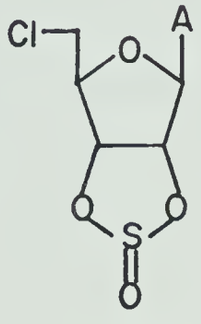
cyclonucleoside formation. 5'-O-Tosyladenosine is considerably more resistent to cyclonucleoside formation than its 2',3'-isopropylidene derivative. In the present study 5'-azido-5'-deoxyadenosine (62) was prepared by reaction of 5'-O-tosyladenosine with lithium azide at 80° for 45 minutes. Only a small smount of cyclonucleoside was formed and it was removed when the product was crystallized from water. Oxidation-reduction of this azido product gave the ring-opened analogue 5'-azido-5'-deoxyadenosine$_{ox\text{-}red}$ (63). 5'-O-Tosyladenosine$_{ox\text{-}red}$ was much more stable than 5'-O-tosyladenosine with respect to cyclonucleoside salt formation. No substitution reactions were carried out using this compound since alternate routes to the 5'-substituted analogues were available, but 5'-O-tosyladenosine$_{ox\text{-}red}$ should be able to withstand fairly severe reaction conditions.

5'-Chloro-5'-deoxyadenosine (64) was prepared directly from adenosine using thionyl chloride in pyridine/acetonitrile.[126] The initial product of this reaction is the cyclic sulfinate of 5'-chloro-5'-deoxyadenosine (70) which is collected as a precipitate and hydrolysed to the desired product in methanol/conc. ammonia (9:1). 5'-Chloro-5'-deoxyadenosine$_{ox\text{-}red}$ (65) was prepared by oxidation-reduction of 64. Purification of the oxidized-reduced nucleosides was primarly a matter of removing iodate and borate salts. During the preparation of


HO
A
O
HO
OH
SOCl2
Cl
64
1) NaIO4
2) NaBH4
65
LiEt3BH
TsO
59
H+
H3C
68
69
60
LiN3
N3
62
63
RaNi
hydrazine
61
H2N
66
67

Scheme VIII

5'-chloro-5'-deoxyadenosine$_{ox-red}$, desalting was accomplished by adsorbing the nucleoside from the reaction mixture onto charcoal. The charcoal was washed thoroughly with water and then the product was eluted with 50% acetonitrile/water. This method is more convenient than column chromatography, which was used to purify several other oxidized-reduced compounds.



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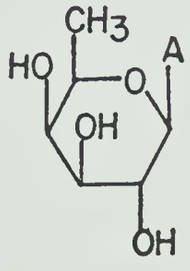


71

5'-Deoxyadenosine (68) was synthesized by the reaction of lithium triethylborohydride ("super hydride") with 5'-chloro-5'-deoxyadenosine.[127] Oxidation-reduction of this product gave 5'-deoxyadenosine$_{ox-red}$ (69). When the volume of the oxidation-reduction mixture was reduced by evaporation, the product crystallized and no further purification was necessary. This compound was prepared by Lerner and Rossi[54] by oxidation-reduction of 9-β-D-fucopyranosyl adenine (71).

5'-Amino-5'-deoxyadenosine (66) and 5'-amino-5'-deoxyadenosine$_{ox-red}$ (67) were prepared by Raney nickel/hydrazine reduction of the 5'-azido compounds 62 and 63

respectively. It was necessary to filter the reaction mixtures several times to remove fine nickel particles but no further purification was needed.

ACID CATALYSED HYDROLYSIS.

All hydrolyses were carried out in 1 *M* HCl in 3:2 dioxane/water. All the nucleosides were readily soluble in this mixture. The hydrolyses were determined at 50°C except for those of compounds 51, 53, 54, and 69. These compounds hydrolysed too quickly at 50°C and were therefore studied at 0°C. The solutions were 0.025 M in nucleoside so that the acid concentration was essentially constant and pseudo-first order kinetics were observed.

In a first order reaction the substrate "A" is converted to the product "P" in the rate determining step, which is characterized by the rate constant "k". At time t = 0 let the amount of A be "a" and the amount of P is 0. At any time t let the amount of P be "x", so that the amount of A is a-x. The rate of disappearance of A is described by equation (1). Integration

$$A \xrightarrow{k} P$$

$$\frac{dx}{dt} = k(a-x) \qquad (1)$$

$$\ln(a-x) = -kt + \ln a \qquad (2)$$

and rearrangement of (1) gives equation (2). Ln a is a

constant so that a plot of ln(a-x) vs t yields a straight line with slope -k and with the intercept on the ln(a-x) axis being ln a. Plots of this type were used in the present work to determine the values of k. It is more meaningful to describe the stability of a nucleoside in terms of its half life, the time for half the nucleoside to be hydrolysed. This value is calculated directly from k. Equation (2) rearranges to equation (3). When half of the starting material has been hydrolysed, $a-x = \frac{a}{2}$ and $t = \tau$, the half life. Thus equation (3) becomes equation (4) which rearranges to give equation (5).

$$\ln \left(\frac{a}{a-x} \right) = kt \qquad (3)$$

$$\ln \left(\frac{a}{a/2} \right) = k\tau \qquad (4)$$

$$\tau = \frac{\ln 2}{k} = \frac{0.6932}{k} \qquad (5)$$

The kinetic data were treated by three methods. Manually plotting the data on a graph, and treatment of the data with a least squares programme both give the best straight line through the data points and tend to ignore "bad" points. The third method involved averaging the instantaneous rate constants between each two points. This procedure tends to emphasize bad points so that while the error limits provided by the least squares programme were plus or minus a few percent the standard deviation values determined from the instantaneous rate

constants were ±20-30% for the best plots and ±200% for the worst ones. The procedure used to carry out the hydrolyses is relatively crude, particularly since it involves handling the samples, and large error limits are to be expected. However, a small number of bad points that could be ignored is also to be expected and such points are obvious on inspection of the graphs. Therefore, the large error limits determined from the instantaneous rate constants misrepresent the accuracy of the hydrolysis procedure. Since the half life values determined for any compound by the three methods are within a few percent of each other and since the differences in rates between compounds are usually much greater than the error limits, the half life values accurately reflect the order of acid stability. The half life values are presented in Tables II and III.

The largest error limits were always those calculated for the compounds with the longest half lives. It is possible that this is the result of slow side reactions. However, additional UV light absorbing spots were never observed on paper chromatograms of the hydrolysis samples. In addition, these plots tended to be erratic rather than having consistent deviation trends.

An obvious feature of these rate studies was the much faster hydrolysis rates measured with the ring-opened nucleosides. This observation was expected. Hydrolysis

TABLE II

Half life values determined by three methods - each value is an average of three runs

COMPOUND	graph	least squares	instantaneous constants	COMPOUND	graph	least squares	instantaneous constants
adenosine	102 min	100 min	101 min	5'OTsA$_{ox-red}$	4.1 hr	4.1 hr	4.8 hr
A$_{ox-red}$	7.2 min	7.2 min	6.7 min	5'Cl A$_{ox-red}$	1.5 hr	1.4 hr	1.4 hr
A$_{ox-red}$ @ 0°	301 hr	315 hr	319 hr	5'N$_3$ A$_{ox-red}$	1.0 hr	1.0 hr	1.1 hr
2'OTsA$_{ox-red}$	11.4 hr	11.3 hr	11.5 hr	5'NH$_2$ A$_{ox-red}$	4.7 hr	4.6 hr	4.6 hr
2'Cl A$_{ox-red}$	2.7 hr	2.3 hr	2.5 hr	5'NH$_2$A	84 hr	-	84 hr
2'BrA$_{ox-red}$	1.6 hr	1.6 hr	1.7 hr	5'dA$_{ox-red}$ @ 0°	53 hr	-	54 hr
2'IA$_{ox-red}$ @ 0°	133 hr	137 hr	144 hr				
2'N$_3$A$_{ox-red}$	48 min	49 min	50 min				
2'NH$_2$A$_{ox-red}$	2.7 hr	2.6 hr	2.5 hr				
2'dA$_{ox-red}$ @0°	68 min	69 min	65 min				

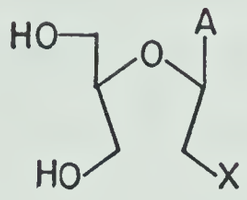
(continued...)

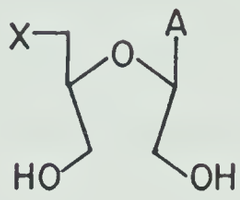
TABLE II (continued)

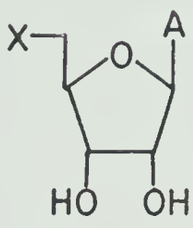
1'-2' unsatd $A_{ox\text{-}red}$ @ 0°	7.3 min	7.4 min	6.6 min
5'OTsA	23 hr	18 hr	19 hr
5'N_3A	14 hr	13 hr	14 hr
5'Cl A	22 hr	22 hr	24 hr
5'dA	25 min	25 min	26 min

TABLE III

Half life value in hours at 50°C - *denotes half life at 0°C

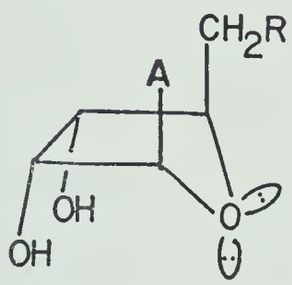






X=OH	0.1,301*	0.1,301*	1.7
OTs	11	4.1	23
Cl	2.7	1.5	22
Br	1.6	-	-
I	133*	-	-
H	1.1*	53*	0.4
N_3	0.8	1.0	14
NH_2	2.7	4.7	84

of the glycosyl bond of nucleosides with an intact ribose ring should proceed via an $_{o}E$ (O1'exo, P ≅ 370°)[128] conformation (structure 72) in which a lone pair of electrons on the ring oxygen is oriented anti-periplanar to the adenine leaving group and can assist glycosyl bond cleavage. Robins and MacCoss[129] confirmed that 3',5'-cAMP (73), in which the trans-fused ring system prevents the $_{o}E$ conformation entirely, is much more stable to acid than 5'AMP. In fact they observed that glycosyl bond hydrolysis



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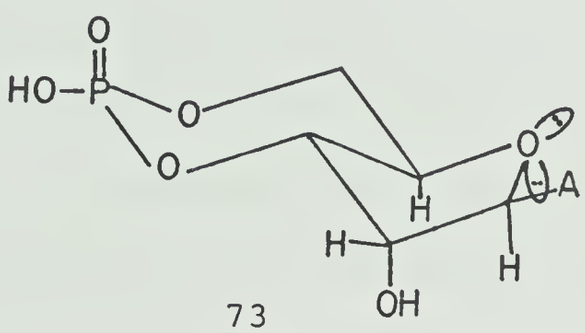
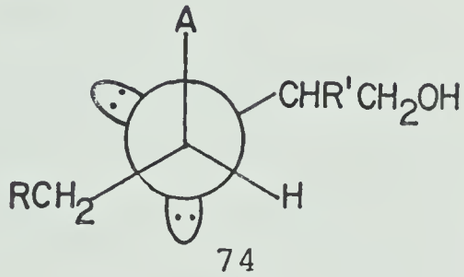


73

of 73 proceeded by initial hydrolysis of the cyclic phosphate to 3'- or 5'-AMP which then underwent glycosyl bond hydrolysis in the normal manner. They observed that the 3',5'-cyclic phosphate of adenosine$_{ox-red}$ is hydrolyzed faster than 5'AMP (intact ribofuranase ring). An $_{o}E$ conformation promotes a 1,3-interaction of adenine and the 5' substituted methyl group and eclipsing of the 3' hydroxyl and the 2' substituent. This conformer should therefore occupy but a small fraction of the total population. In their survey of the crystal structures of 60

nucleosides, Altona and Sundaralingam[128] found that almost all were either in type N (3'endo, P = 0-36°) or type S (2' endo, P = 144-180°) conformations. None were in the $_{o}E$ conformation. They and others[130] have argued that the crystal state conformers also predominate in solution. The transition state leading to glycosyl bond cleavage, which is thought to be the rate determining step in the hydrolysis reaction, must resemble the $_{o}E$ conformation. This is a relatively high energy state. On the other hand, the oxygen lone pairs of ring-opened adenosine derivatives are oriented anti-periplanar to adenine in two of the three staggered rotamers about the C1'-O bond (structures 74 and 75) and should be highly populated conformers. Glycosyl bond cleavage should therefore proceed



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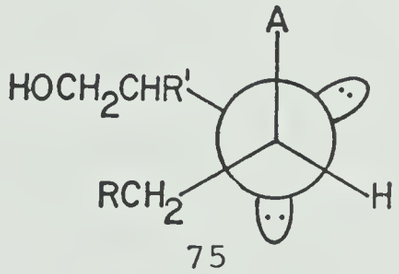

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via a relatively lower energy transition state. Thus a ring-opened nucleoside should hydrolyse much faster than the corresponding ring-closed compound, as was observed in all cases in this study.

Since the orientation of the oxygen lone pairs is important in determining hydrolysis rates, certain inter- and intramolecular interactions which influence conformational preferences should be considered. H-bonding between the 5' hydroxyl group and N3 of adenine has been discussed previously in this thesis. H-bonding between the 2' hydroxyl group and N3 has been observed in organic solvents[115] and there is some indirect evidence for it in aqueous solutions as well.[131] However, the evidence is not conclusive and the effects observed are very small. Furthermore, Zoltewicz has claimed[89] that species with diprotonated bases dominate the kinetics of these hydrolysis reactions. The N3 of such species or even monoprotonated species, would be a poor electron donor. Such intramolecular H-bonding interactions would therefore be considered to have negligible effects on hydrolysis rates. Intramolecular base stacking has been observed in neutral aqueous solution.[132] However, in the hydrolysis solutions, virtually all of the bases would be protonated and would therefore repel each other. As well, Ts'O states[133] that no H-bonding is observed between nucleoside bases in aqueous solution.

The ring-opened nucleosides should be much more conformationally flexible than normal intact-ring nucleosides. They could adopt conformations in which substituents are oriented favorably for intramolecular H-bonding, but the associated loss of entropy would be

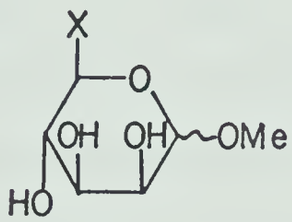
expected to be too large. At the elevated temperatures employed in most of the hydrolysis reactions, entropy considerations are even more important. Intramolecular H-bonding between hydroxyl groups is possible when the sugar ring is opened, but prior studies have shown that intramolecular H-bonding between the 5' and 2' hydroxyl groups of arabinonucleosides is unimportant except at high pH.[122,134] Therefore, under the present hydrolysis conditions the ring-opened nucleosides should be considered to be fully solvated and almost freely rotating. The above mentioned steric interactions and eclipsing of substituents in normal nucleosides should not affect hydrolysis rates of the ring-opened compounds. Evidence for the conformational freedom of the latter compounds is provided by CD spectroscopy. The magnitude of the Cotton effects in the spectra of adenosine dialdehyde[37] and adenosine$_{ox\text{-}red}$[135] at room temperature is much smaller than those in the spectrum of adenosine. This suggests much greater conformational freedom about the glycosyl bond.

As discussed in the introduction, evidence to date is overwhelmingly in favor of an A-1 mechanism in glycosyl bond hydrolysis. This mechanism involves formation of an oxygen stabilized carbonium ion at C1' in the rate determining step. Therefore, electron withdrawing substituents on the sugar, particularly withdrawing

substituents at the adjacent 2' position, should decrease the rate of hydrolysis by destabilizing the formation of this carbonium ion. Hydrolysis rates cannot be correlated quantitatively with electron withdrawing ability because there is no numerical scale of the withdrawing power of chemical groups. Electronegativity is a commonly used index, but it is a property of theoretically isolated atoms. Furthermore, there are several scales of electronegativity whose values vary depending on the method used to derive them. Taft introduced his σ^* parameter in the early 1950's as a measure of the electronic effects of substituents on aliphatic systems, but there have been criticisms of the use of these values and it appears that they are not applicable to this study. This subject has been reviewed by Shorter.[136]

A striking feature of the rate data is the large stabilizing influence exerted by electron withdrawing 5' substituents, which are separated from the glycosyl reaction centre by four σ-bonds. Electronic effects at the ring oxygen are important since this atom is involved in resonance stabilization of the carbonium ion at C1'. This oxygen is separated from the 5' substituent by three σ-bonds, but effects from such a distant group have been observed in carbohydrate chemistry. For instance it was observed some time ago that the rates of hydrolysis of the 5-substituted α- and β- methyl mannopyranosides (76) were

in the order X = H > CH_3 > CH_2OH > $CHOHCH_2OH$[137] and that the rates for the substituted acetals 77 were in the order X = H > CH_2OH > COOH > COOEt.[138] Subsequently the mechanism of glycopyranoside hydrolysis was extensively investigated. Most workers agreed on an Al mechanism involving a cyclic



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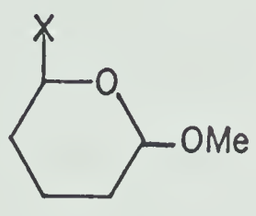

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oxy-carbonium ion.[139-144] However, there was disagreement as to whether the rates correlated with the polarity of the 5-substituent.[142,145-147] Some investigators stressed the importance of conformational factors, particularly the ease of rotation about the C2-C3 and C4-C5 bonds, since formation of the cyclic oxy-carbonium ion causes some eclipsing of the substituents on these carbons.[142,145,148] Timell and coworkers[146] found no correlation of rates either with the polarity of the 5 substituent or with its size. They came to the conclusion ultimately reached by most other workers that the rates result from complex combinations of electronic and steric factors and that it is difficult to definitely establish a mechanism.[142,145,149,150] As Timell has pointed out,[151]

this problem was compounded by the serious disagreement between rate constants published by various workers for identical glycosides.

In the hydrolysis of the 5'-substituted ring-opened nucleosides prepared in this study, eclipsing of substituents upon formation of the oxy-carbonium ion at C1' is not a factor. In view of the conformational freedom of these molecules, the effects of the 5'-substituents must be predominantly electronic. It is interesting and somewhat surprising that the substituents provide a stabilizing effect of the same general order of magnitude whether located at the 2' or 5' position.

In general within each of the three series of compounds prepared in this study, acid stability correlates with the electron withdrawing effect of the substituents. Compounds substituted with strongly withdrawing groups such as amino (which is fully protonated in the acidic hydrolysis solution) are the most stable whereas the deoxynucleosides are the least stable. The same order of stabilities was observed for both 5'-substituted series: NH_3^+ > T_sO > Cl > N_3 > OH > H. (A note must be made here regarding 5'-O-tosyladenosine. From the values given in Table II it appears to have a shorter life than 5'-chloro-5'-deoxyadenosine. These values were obtained using all the data points. The plots of ln (% nucleoside) vs. time for 5'-O-tosyladenosine deviated considerably from linearity.

In particular, samples analysed after long reaction times appeared to give excessively large values of the extent of hydrolysis, probably as a result of slow side reactions. The samples analysed after long reaction times were yellow coloured which also indicates that side reactions were occurring. This yellow colour was not observed in samples of any other compound. The samples of 5'-O-tosyladenosine analysed at shorter reaction times gave a reasonable linear plot. From these data points it appears that the half life of acid hydrolysis of this compound is several hours longer than that of 5'-chloro-5'deoxyadenosine.) The 5' position is far removed spatially from the reaction centre, and this order of stabilities must reflect the electronic effects of these substituents in the hydrolysis process. It is somewhat surprising that chlorine has a much greater stabilizing effect than hydroxyl since the electronegativities of chlorine and oxygen are roughly equal. This may be due to solvation effects. Solvation will remove electron density from chlorine and make it more withdrawing. The hydroxyl group can act as either a donor or acceptor in H-bonds to the solvent and the net effect may be increased electron density on the oxygen.

The order of stabilities in the 2' substituted series is $T_sO > NH_3^+ \simeq Cl > Br > N_3 > OH > I > H$. Iodine and hydrogen have approximately equal electronegativities but the 2'-iodo analogue is much more stable than

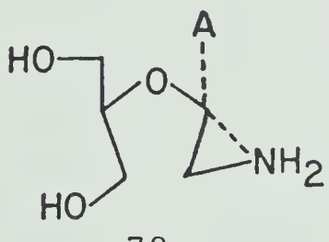
2'-deoxyadenosine$_{ox\text{-}red}$. Iodine is very polarizable and its withdrawing ability should not be affected very much by solvation. The large iodine atom possibly interferes with solvation of the carbonium ion developing at C1'. Garrett[93] attributed the slightly greater stability of 2'-O-methyl adenosine (compared with adenosine) to steric interference with solvation at C1'. Sporns[152] rationalized the greater stability of 2'-O-benzyl adenosine relative to 2'-O-methyl adenosine in the same way. The fact that 2'-iodo-2' deoxyadenosine$_{ox\text{-}red}$ and 2'-deoxyadenosine$_{ox\text{-}red}$ were hydrolysed at a temperature (0°C) where they would be more restricted conformationally may enhance this effect. It is most likely because of steric interference with solvation that 2'-O-tosyladenosine is the most stable compound in the 2'-substituted series. It is more stable than 2'-amino-2'-deoxyadenosine$_{ox\text{-}red}$ in which the 2' substituent carries a formal positive charge. Structures involving overlap of adenine and the aromatic ring of the tosyl group may be involved. Sporns discounted overlap of the base and aromatic 2' substituents as a factor in the hydrolysis of intact-ring adenine nucleosides. Such overlap effects were identified by means of UV and CD spectral studies.[152] The flexibility of 2'-O-tosyladenosine$_{ox\text{-}red}$ allows it to adopt conformations giving extensive overlap as indicated by molecular models. However, it is difficult to predict whether such overlap

with the accompanying loss of entropy would occur during the hydrolysis process at 50°C. The lower extinction coefficient of 2'-O-tosyladenosine$_{ox-red}$ relative to that of 5'-O-tosyladenosine in UV spectra measured in the hydrolysis solvent mixture at room temperature may be due to overlap of the base and tosyl rings.

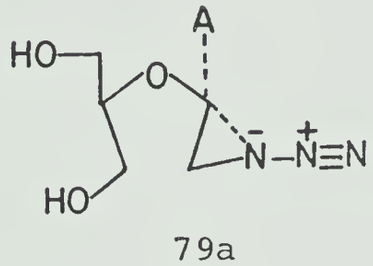
As would be expected considering electronic and steric effects, 2'-O-tosyladenosine$_{ox-red}$ is more stable than 5'-O-tosyladenosine$_{ox-red}$, 2'-chloro-2'-deoxyadenosine$_{ox-red}$ is more stable than 5'-chloro-5'-deoxyadenosine$_{ox-red}$, and 2'-deoxyadenosine$_{ox-red}$ is less stable than 5'-deoxyadenosine$_{ox-red}$. There are, however, some anomalous results which are difficult to explain. 2'-Azido-2'-deoxyadenosine$_{ox-red}$ is less stable than 5'-azido-5'-deoxyadenosine$_{ox-red}$. The error limits involved in the rate measurements are large enough that this ordering may not be significant, but it is surprising that the stabilities are similar. Even more surprising is the fact that 2'-amino-2'-deoxyadenosine$_{ox-red}$ is hydrolysed much more rapidly than 5'-amino-5'-deoxyadenosine$_{ox-red}$, and at approximately the same rate as 2'-chloro-2'-deoxyadenosine$_{ox-red}$. Garrett[93] discussed the possibility that H-bonding with N3 of adenine could decrease the electron withdrawing effect of a 2'-substituent. The 2'-amino group should be extensively H-bonded. However, the species with a diprotonated adenine is thought to dominate the kinetics and N3 of a deprotonated

adenine would be a poor electron donor. Another possibility is that diprotonation of the base decreases the protonation of the amino group but this effect is difficult to evaluate at pH 1.

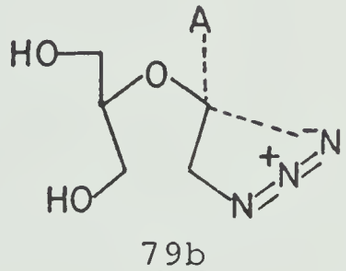
It is possible that the 2'-amino- and 2'-azido- groups increase the rate of hydrolysis by somehow facilitating solvation at C1' but it is difficult to visualize reasonable structures in support of this possibility. Another rationalization is that these substituents themselves assist the glycosyl bond cleavage, which is thought to be the rate determining step, by means of the aziridine-like and triazole-like structures 78 and 79. However, the negative charge on the azide group is delocalized over two



78



79a



79b

nitrogens and the neutral amine species would represent an extremely small fraction of the population at pH 1. In addition, these structures would involve a decrease in entropy. Therefore, the unusually rapid hydrolyses of 2'-amino-2'-deoxyadenosine$_{ox\text{-}red}$ and 2'-azido-2'-deoxyadenosine$_{ox\text{-}red}$ must be regarded as anomalous results.

Compound 54 was the most acid labile of all the compounds presently studied. Hydrolysis of this N,O-ketene acetal probably proceeds by addition of water across the double bond.

EXPERIMENTAL

GENERAL PROCEDURES.

Melting points were determined on a Reichert microstage apparatus and are uncorrected. Proton NMR spectra were recorded on a Varian HA-100 instrument in perdeuterated Me_2SO with Me_4Si as an internal standard. Peak assignments were made with the aid of spin-spin decoupling and D_2O exchange spectra. UV spectra were recorded on a Cary 15 spectrometer. Mass spectra were obtained by the mass spectroscopy laboratory of this department on an AEI MS-50 instrument using direct probe sample introduction at 150-200°. All peaks quoted gave satisfactory exact mass measurements in accordance with the structures assigned to them. Elemental analyses were determined by the micro-analytical laboratory of this department or by Schwartzkopf Microanalytical Laboratory, Woodside, New York.

Thin layer chromatography was performed on Eastman chromatogram sheets (silica gel No. 13181, indicator No. 6060) in the solvent systems indicated and monitored with ultraviolet light (254 nm). Silica gel column chromato-graphy was performed on JT Baker No. 3405 silica gel (60-200 mesh) and anion exchange chromatography was carried out on Dow Chemical Dowex 1x2 hydroxide form.

The carbon used was Barnebey-Cheney AU-4 charcoal. It was conditioned in the following way. The charcoal was washed with methanol and then chloroform. After drying it was refluxed with 1 M aqueous HCl, with the acid

solutions being replaced periodically until it remained colourless. The charcoal was washed with water to neutrality and then refluxed with 10% aqueous NaOH, again with periodic replacement of the solution until it remained colourless. The charcoal was then washed with water to neutrality, with methanol, and finally with chloroform before it was air dried at room temperature.

The hydrogenation catalyst was 5% palladium on charcoal purchased from Terochem Laboratories Ltd., Edmonton, Alberta. Raney Nickel was purchased from W. R. Grace and Co., South Pittsburg, Tennessee. The p-nitrophenyl esters of CBZ-L-phenylalanine and CBZ-L-leucine were purchased from Sigma Chemical Co., St. Louis, Missouri. Adenosine was purchased from Kohjin Company Ltd., Tokyo.

All solvents used were reagent grade. Methanol, methylene chloride, and chloroform were distilled before use. Pyridine and DMSO were dried by reflux and distillation over calcium hydride. Acetonitrile and DMF were dried by reflux and distillation over diphosphorous pentoxide. All dried solvents were stored over Davison 4 Å molecular seives purchased from Fisher Scientific Company, Fair Lawn, New Jersey.

Evaporations were carried out using a Buchler rotary evaporator equipped with a dry-ice condenser, using either water aspirator or oil pump vacuum.

Diffusion crystallization[153] was carried out using alcohol/ether unless specified otherwise. A flask containing a concentrated solution of the nucleoside in methyl or ethyl alcohol was placed in a desiccator over ether (in which the nucleoside is insoluble). Crystallization was allowed to proceed at room temperature for one to five days and the product collected without cooling.

Crystallization from ethyl acetate was carried out by first dissolving the nucleoside in a small volume of water since it was very difficult to dissolve it directly in ethyl acetate. A large volume of ethyl acetate was then added to this solution. Boiling the mixture removes the water as an azeotrope and then reduces the volume of ethyl acetate so that the product crystallizes on cooling.

Unless specified otherwise, the oxidation-reduction procedure was as follows. The nucleoside was dissolved or suspended in water at a concentration of about 0.02 M. To this was added 1.1 equivalents of sodium meta-periodate. Any suspended nucleoside dissolved within a few minutes. The solution was stirred at room temperature while protected from light for one hour, then checked for completeness by tlc on silica gel with 10% methanol/chloroform. Two equivalents of sodium borohydride were added and this solution was stirred at room temperature for one hour. The reaction was then checked for completeness using the same tlc system. Excess borohydride was destroyed by

addition of acetic acid to pH 7. Glacial acetic acid was used for large scale reactions and 1 M acetic acid was used for small scale reactions. If a pH of 5 or lower was reached accidentally, formation of elemental iodine was observed. No nucleosidic byproducts were observed in these reactions. Desalting was carried out as indicated for each compound.

ACID CATALYSED HYDROLYSES.

All hydrolysis solutions were 1 *M* HCl in 3:2 dioxane/water. The concentration of nucleoside was 0.025 M. A weighed sample of nucleoside was dissolved in 3:1 dioxane/water and this solution was cooled in ice before mixing with one quarter its volume of 5 M HCl. 20 µl samples were injected into closed end capillary tubes and then the open ends of the tubes were sealed. The capillary tubes were kept in an ice bath at all times until they were immersed in a 10 gallon oil bath equipped with an electric heater and an electric stirring motor. The oil bath was allowed to equilibrate for several days before rate determinations were made and the temperature was never observed to vary from the specified 50°C by more than 0.5°. At regular intervals capillary tubes were removed from the oil bath and immediately injected into 25 µl aliquots of saturated sodium bicarbonate solution. When hydrolyses were carried out at 0°C, the entire hydrolysis

solution was kept in one vial immersed in an ice/water bath in the refrigerator. At regular intervals 20 µl samples were withdrawn and neutralized in the same way.

The neutralized samples were analysed by descending chromatography on Whatman No. 1 paper using t-butanol/methyl ethyl ketone/water/conc ammonia (4:3:2:1) as solvent system. Products were detected by UV light (254 nm) and the spots were cut out in standard size circles (5.5 cm diameter). Products were eluted by cutting up the circles and immersing the pieces in 4 ml aliquots of 0.1 M HCl for two hours. Hydrolysis of the nucleoside analogue in this eluting solution was taken to be negligible for all compounds except the very labile 2'-deoxyadenosine$_{ox\text{-}red}$ (53) and its 1'-2' unsaturated analogue (54). For these compounds complete hydrolysis to adenine was assumed. The UV absorption of each aliquot was measured without dilution, a blank value for an identically treated circle of paper was subtracted, and the absorption values were converted to numbers of moles using the known extinction coefficients. Each compound was hydrolysed in three separate runs, with each run consisting of nine capillary samples.

For each sample the number of moles of unhydrolysed nucleoside and the number of moles of adenine were calculated. The extent of hydrolysis was expressed as the percentage of nucleoside remaining unhydrolysed. In

the graphic treatment of the data a plot of ln (% nucleoside) vs. time was made and the best straight line was drawn through the data points by visual inspection. Occasionally an obvious bad point was neglected. The best straight line was also determined on a Hewlett-Packard HP 65 programmable calculator using the Stat 1-05A and Stat 1-22A programmes. Stat 1-05A enters and sums the set of data points $\{(x_i, y_i),\ i = 1,2,...9\}$ and Stat 1-22A fits a straight line $y = a_0 + a_1 x$ to these data points by the least squares method.

$$a_1 = \frac{\Sigma x_i y_i - \frac{\Sigma x_i \Sigma y_i}{n}}{\Sigma x_i^2 - \frac{(\Sigma x_i)^2}{n}}$$

$$a_0 = \bar{y} - a_1 \bar{x} \text{ where } \bar{x} = \frac{\Sigma x_i}{n} \text{ and } \bar{y} = \frac{\Sigma y_i}{n}$$

This programme also computes the correlation coefficient r; where

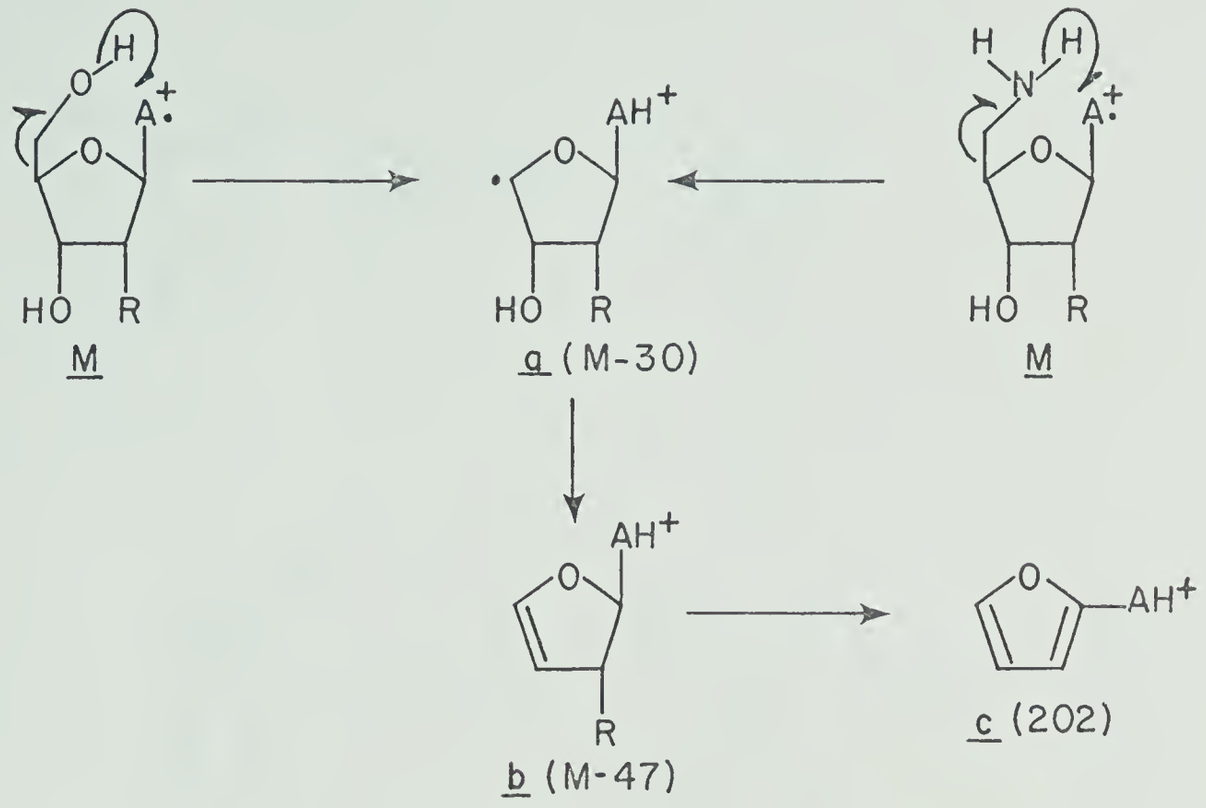
$$r^2 = \frac{[\ \Sigma x_i y_i - \frac{\Sigma x_i \Sigma y_i}{n}]^2}{[\Sigma x_i^2 - \frac{(\Sigma x_i)^2}{n}\ ][\Sigma y_i^2 - \frac{(\Sigma y_i)^2}{n}\ ]}$$

The third method of data treatment involved calculation of the values of k, the rate constant, between each two data points. The overall rate constant was obtained by averaging these instantaneous rate constants. The deviation of each instantaneous rate constant from the average

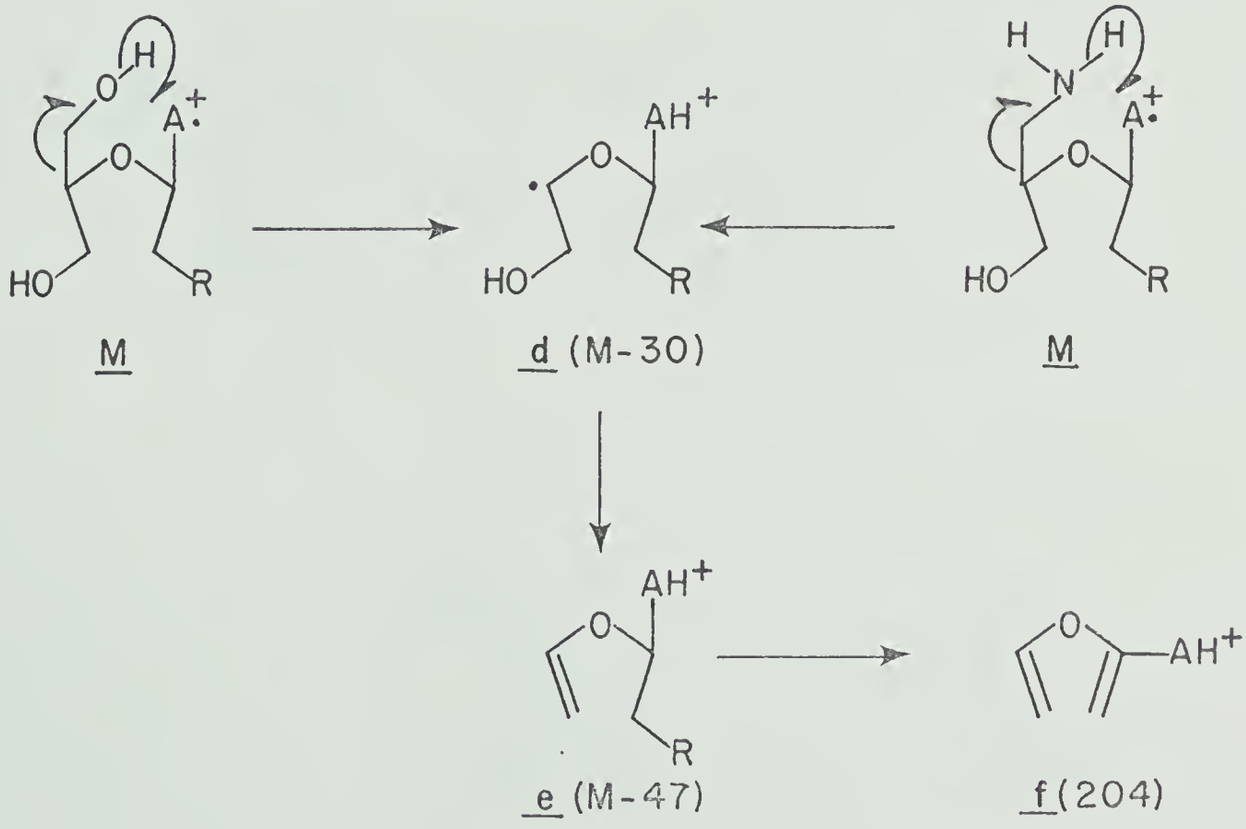
was calculated and from these values the average deviation was calculated, as an indication of error limits.

Mass spectral data. The mass spectral fragmentation of adenine nucleosides has been studied by McCloskey and coworkers.[154] Schemes IX and XI illustrate some fragmentation pathways leading to commonly observed ions, as proposed by these workers. Scheme X illustrates pathways for ring opened nucleosides analagous to those shown in Scheme IX.

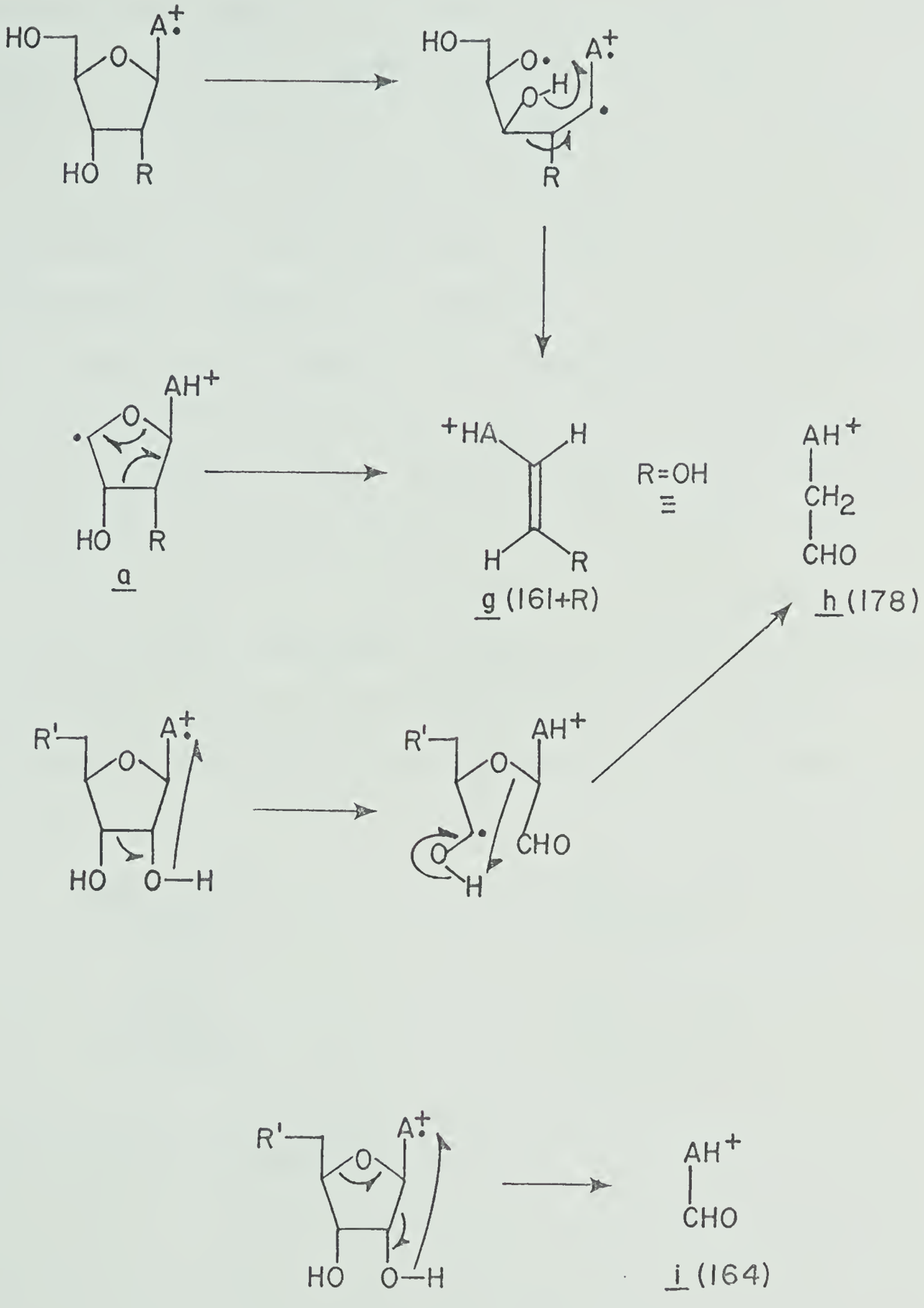
The M^+ ion was observed in the spectra of all of the present compounds except those substituted with a tosyl group. Ion d was observed in the spectra of all the 2'-substituted compounds. It has diagnostic value since it carries the 2' substituent. Ion e was rarely observed and was never intense. Ion f was not observed. Ions a and d were formed from the 5'-amino substituted compounds 66 and 67 respectively by loss of CH_2NH as shown in Schemes IX and X. An ion with mass M-30 might be expected in the spectra of the 5'-substituted ring-opened compounds since the 3'-hdyroxymethyl group could be lost by a mechanism analogous to the one depicted in Scheme X. However, such an ion was observed only in the spectrum of 5'-deoxyadenosine$_{ox-red}$. SchemeXI illustrates the formation of ions g and h, which are numerically identical (tautomers) when R = OH. Similar pathways must be involved



Scheme IX

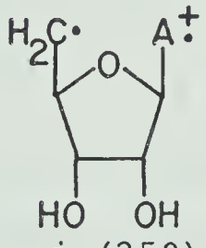


Scheme X

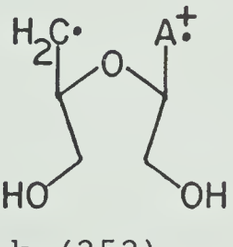


Scheme XI

in the ring-opened nucleoside series since ion g was observed in the spectra of all of the 2'-substituted compounds. It is a valuable diagnostic ion since it carries the 2' substituent. Ion h was present as a fairly large peak in the spectra of all 5' substituted compounds, but appeared as a very small peak in the spectra of the 2'-substituted compounds, as expected. Ion i was an intense peak in the spectra of all compounds. Sporns[152] also observed formation of this ion from 2'-substituted nucleosides (intact-ring). Proton transfer from a position other than the 2' position must be involved. Ions AH^+ (135) and AH_2^+ (136) were observed in the spectra of all of the present compounds and one of these was usually the mass spectral base peak (100% relative intensity). Loss of the 5' substituent to form ion j or ion k was a



j (250)



k (252)

commonly observed fragmentation.

SYNTHESES

Preparation of adenosine dialdehyde (14). To 5.36 g (20 mmole) of adenosine (1) suspended in 80 ml of water was added 4.44 g (22 mmole) of sodium periodate. The colourless solution which formed in a few minutes was stirred at room temperature in the dark. A white precipitate was observed after two hours. The mixture was allowed to stand for two days and the precipitate was collected by filtration. A second crop was collected by allowing the filtrate to stand for two days. Total yield was 4.01 g (73%) of 14 as a white powder.

Preparation of adenosine$_{ox\text{-}red}$ (25). To 400 mg (1.5 mmole) of 14 suspended in 75 ml of water was added 114 mg (3.0 mmole) of sodium borohydride and the resulting solution was stirred for one hour at room temperature. The solution was neutralized to pH 7 with acetic acid and then was stirred with 2 g of charcoal until the UV absorption indicated less than 5% of the nucleoside in solution. The charcoal was collected by filtration and the nucleoside eluted by continuous extraction in a soxhlet apparatus with 9:1 ethanol/conc ammonia. Evaporation of the extract gave a white solid which was crystallized from ethanol by diffusion of ether to give 281 mg (70%) of 25: mp 143-145°C; UV (H_2O) max 259 nm (ε 15,300); UV (1 M HCl) max 257 nm (ε 15,000); UV (1 M NaOH) max 260 nm (ε 15,300);

NMR (DMSO-d_6) δ3.18 (m,2,$CH_2$3'), 3.50 (m,3,$CH_2$5' and CH4'), 3.88 (t,2, J = 6Hz, $CH_2$2'), 4.43 (t,1, J = 6Hz, OH 5'), 4.68 (t,1,J=5Hz, OH 3'), 5.12 (t, 1, J = 6Hz, OH 2'), 5.92 (t, 1, J = 6Hz, CH 1'), 7.22 (s, 2, NH_2), 8.18 (s, 1, H2), 8.28 (s, 1, H8); ms (200°) m/e 269.1138 (calcd for M^+ 269.1124, 1%), 178 (11, h), 164 (17, i), 136 (27, AH_2), 135 (100, AH).

Anal. Calcd for $C_{10}H_{15}N_5O_4$: C, 44.60; H, 5.62; N, 26.01. Found: C, 44.58; H, 5.45; N, 26.25.

Preparation of 2',3'-dideuteroadenosine$_{ox\text{-}red}$ (38). The procedure for the preparation of adenosine$_{ox\text{-}red}$ was repeated using sodium borodeuteride in place of sodium borohydride to give a 70% yield of crystalline 38: mp 144-145°C; NMR (DMSO-d_6) δ3.18 (m, 1, CHD 3'), 3.40 (m overlapped with H_2O, CH_2 5' and CH 4'), 3.85 (br d, 1, CHD 2'), 4.42 (br t, 1, OH 5'), 4.64 (br d, 1, OH 3'), 5.08 (br d, 1, OH 2'), 5.87 (d, 1, J = 6Hz, CH 1'), 7.18 (s, 2, NH_2), 8.14 (s, 1, H2), 8.24 (s, 1, H8); ms (200°) m/e 271.1254 (calcd for M^+ 271.1250, 2%), 180 (9, h), 179 (13, h), 178 (1, h), 164 (19, i), 136 (28, AH_2), 135 (100, AH).

Anal. Calcd for $C_{10}H_{13}D_2N_5O_4$: C, 44.29; H, 5.62 for a gc volume analysis technique; N, 25.82. Found: C, 43.94; H, 5.62; N, 25.88.

Preparation of 3',5'-ethoxymethylideneadenosine$_{ox-red}$ (39). To 4.0 g (15 mmole) of 25 dissolved in 800 ml 3:5 DMF/dioxane was added 10 g (60 mmole) trichloroacetic acid and 8 ml (45 mmole) triethylorthoformate. The solution was stirred at room temperature for one hour, then neutralized with saturated sodium bicarbonate solution and the resulting precipitate removed by filtration. The filtrate was evaporated to dryness and the residue was adsorbed onto 60 g of silica gel by repeated evaporation from methanol. This silica gel was loaded onto the top of a 400 g dry packed silica gel column and the nucleoside was eluted with 2 l 5% methanol/methylene chloride followed by 2 l 10% methanol/methylene chloride. Triethylamine (1 ml/litre) was added to the solvent to prevent deblocking on the column. Evaporation of the appropriate fractions gave 3.64 g (75%) of 39 as a white powder: mp 74-80°C; NMR (DMSO-d_6) δ1.10 (t, 3, J = 7Hz, CH_3), 3.51 (m, 4, CH_2 5' and CH_2 ethyl), 3.92 (m, 5, CH_2 2', CH_2 3', and CH 4'), 5.18 (t, 1, J = 5Hz, OH 2'), 5.23 (s, total with δ5.29 signal = 1, CH orthoester), 5.29 (s, CH orthoester), 5.79 (t, 1, J = 6Hz, CH 1'), 7.24 (s, 2, NH_2), 8.15 (s, 1, H2), 8.30 (s, 1, H8).

Anal. Calcd for $C_{13}H_{19}N_5O_5$: C, 47.99; H, 5.89; N, 21.53. Found: C, 47.73; H, 6.06; N, 21.56.

Preparation of 3',5'-ethoxymethylidene-2'-O-tosyladenosine$_{ox-red}$ (42). A solution of 3.0 g (9.3 mmole) of 39 in 350 ml dry pyridine was prepared by warming and then cooled to -20°. 9.0 g (46.5 mmole) of tosyl chloride was added and the resulting solution was allowed to stand overnight at -10°. The solution was poured into 600 ml of cold saturated sodium bicarbonate solution and this mixture was extracted with four 600 ml portions of methylene chloride. The combined methylene chloride layers were washed with water and then reduced to a small volume, which was passed through decolourizing charcoal to remove the yellow colour. Evaporation gave a white foam which was evaporated to dryness from methanol to give a white powder which was easier to collect. Crystallization from ethanol by diffusion of pentane gave 3.23 g (73%) of 42 in four crops: mp 148-156°C; NMR (DMSO-d_6) δ1.09 (t, 3, J = 7Hz, CH_3 ethyl), 2.38 (s, 3, CH_3 tosyl), 3.2-4.2 (unresolved, 7, CH_2 3', CH 4', CH_2 5' and CH_2 ethyl), 4.61 (m, 2, J = 5Hz, CH_2 2'), 5.22 (s, total with δ5.29 signal = 1, CH orthoester), 5.29 (s, CH orthoester), 6.04 (t, 1, J = 5Hz, CH 1'), 7.28 (s, 2, NH_2), 7.34 (d, 2, J = 8Hz, CH aromatic), 7.64 (d, 2, J = 8Hz, CH aromatic), 8.04 (s, 1, H2), 8.24 (s, 1, H8).

Preparation of 2'-O-tosyladenosine$_{ox-red}$ (43). Compound 42 was refluxed in ethanol/trace acetic acid for four hours. The solution was evaporated to dryness and the

residue was dissolved in a small volume of ethanol. On standing 43 precipitates as a white powder: mp 158-168°C; UV (H_2O) max 262 nm (ε 13,300) max 230 nm (ε 13,600); UV (1 M HCl) max 258 nm (ε 14,000), UV (1 M NaOH) max 262 nm (ε 13,700); NMR (DMSO-d_6) δ2.38 (s, 3, CH_3), 3.0-3.6 (unresolved and overlapped with H_2O, CH_2 3', CH 4', and CH_2 5'), 4.44 (t, 1, J = 5Hz, OH 5'), 4.58 (m, 2, CH_2 2'), 4.74 (t, 1, J = 5Hz, OH 3'), 6.10 (t, 1, J = 5Hz, CH 1'), 7.24 (s, 2, NH_2), 7.32 (d, 2, J = 8Hz, CH aromatic), 7.58 (d, 2, J = 8Hz, CH aromatic), 8.04 (s, 1, H2), 8.22 (s, 1, H8); ms (200°) m/e 332 (1, g), 164 (13, i), 136 (24, AH_2), 135 (69, AH).

Anal. Calcd for $C_{17}H_{21}N_5O_6S$: C, 48.22; H, 5.00; N, 16.54. Found: C, 48.16; H, 4.98; N, 16.29.

Preparation of 2'-azido-2'-deoxyadenosine$_{ox\text{-}red}$ (48). A solution of 1.0 g (2.1 mmole) of 42 and 500 mg (10.5 mmole) of lithium azide in 150 ml of dry DMF was stirred at 80° for 24 hours. The solution was evaporated to a syrup which was dissolved in an equal mixture of water and methylene chloride. The layers were separated, the aqueous layer was extracted once with methylene chloride, and the combined methylene chloride layers were washed with water. The organic layer was evaporated to a syrup and chromatographed on a small (25 ml) column of silica gel, eluting first with methylene chloride to remove

residual DMF and then with 10% methanol/methylene chloride. The appropriate fractions were evaporated to a gum which was dissolved and refluxed in ethanol/trace acetic acid for five hours. Evaporation of this solution gave a white solid which was crystallized from ethyl acetate to give 516 mg (94%) of 48 in two crops as fine white needles: mp 160.5-162°C; UV (H_2O) max 260 nm (ε 14,600); UV (1 M HCl) max 256 nm (ε 15,000); UV (1 M NaOH) max 260 nm (ε 15,000); NMR (DMSO-d_6) δ3.1-3.7 (unresolved, 5, CH_2 3', CH 4', CH_2 5'), 3.92 (m, 2, CH_2 2'), 4.46 (t, 1, J = 5Hz, OH 5'), 4.75 (t, 1, J = 5Hz, OH 3'), 6.10 (t, 1, J = 6Hz, CH 1'), 7.25 (s, 2, NH_2), 8.16 (s, 1, H2), 8.33 (s, 1, H8); ms (200°) m/e 294.1181 (calcd for M^+ 294.1189, 3%), 264 (1, d), 203 (1, g), 164 (40, i), 136 (48, AH_2), 135 (100, AH).

Anal. Calcd for $C_{10}H_{14}N_8O_3$: C, 40.81; H, 4.79; N, 38.08. Found: C, 40.71; H, 4.84; N, 37.79.

Preparation of 2'-chloro-2'-deoxyadenosine$_{ox\text{-}red}$ (49). A solution of 280 mg (0.6 mmole) of 42 and 170 mg (3.5 mmole) of lithium chloride in 75 ml of dry DMF was stirred at 80° for 48 hours. The solution was evaporated to a gum which was dissolved in an equal mixture of water and methylene chloride. The layers were separated, the water layer was extracted with methylene chloride, and the combined methylene chloride layers were washed with water.

This organic layer evaporated to a gum which was chromatographed on a silica gel column (2.5 cm x 18 cm) eluting with 2% methanol/methylene chloride to give 81 mg of a white foam. The foam was dissolved in ethanol/trace acetic acid and the solution was refluxed for several hours then evaporated to a glass. This material was chromatographed on a silica gel column (2.5 cm x 18 cm) eluting with 2% methanol/methylene chloride. The appropriate fractions were evaporated to a foam which was crystallized from ethyl acetate to give 50 mg (26%) of 49: mp 158-160°C; UV (H_2O) max 260 nm (ε 15,400); UV (1 M HCl) max 257 nm (ε 15,500); UV (1 M NaOH) max 260 nm (ε 15,900); NMR (DMSO-d_6) δ3.1-3.7 (unresolved, 5, CH_2 3', CH 4', CH_2 5'), 4.22 (m, 2, CH_2 2'), 4.47 (t, 1, J = 5Hz, OH 5'), 4.75 (t, 1, J = 5Hz, OH 3'), 6.10 (dd, 1, J = 6Hz, J = 7Hz, CH 1'), 7.25 (s, 2, NH_2), 8.15 (s, 1, H2), 8.35 (s, 1, H8); ms (200°) m/e 289.0764 (calcd for M^+ 289.0756, 1%), 287.0789 (calcd for M^+ 287.0785, 3%), 259 (0.2, d), 257 (0.6, d), 198 (4, g), 196 (11, g), 164 (14, i), 136 (17, AH_2), 135 (100, AH).

Anal. Calcd for $C_{10}H_{14}N_5O_3Cl$: C, 41.74; H, 4.90; N, 24.24. Found: C, 41.65; H, 4.94; N, 24.09.

Preparation of 2'-bromo-2'-deoxyadenosine$_{ox\text{-}red}$ (50).

A solution of 250 mg (0.5 mmole) of 42 and 230 mg (2.5 mmole) of lithium bromide in 75 ml of dry DMF was stirred

at 80° for 48 hours. The workup was identical to that for the chlorine compound 49 except that instead of chromatographing the product after deblocking it was stirred in methanolic ammonia (pH 9) for one hour at room temperature. Crystallization from ethyl acetate gave 67 mg (36%) of 50: mp 166-167.5°C; UV (H_2O) max 258 nm (ε 14,500); UV (1 M HCl) max 255 nm (ε 14,500); UV (1 M NaOH) max 260 nm (ε 14,700); NMR (DMSO-d_6) δ3.1-3.7 (unresolved, 5, CH_2 3', CH 4', and CH_2 5'), 4.12 (m, 2, CH_2 2'), 4.48 (t, 1, J = 5Hz, OH 5'), 4.76 (t, 1, J = 5 Hz, OH 3'), 6.11 (dd, 1, J = 5Hz, J = 7Hz, CH 1'), 7.26 (s, 2, NH_2), 8.16 (s, 1, H2), 8.36 (s, 1, H8); ms (200°) m/e 333.0249 (calcd for M^+ 333.0260, 1%), 331.0283 (calcd for M^+ 331.0280, 1%), 303 (0.1, d), 301 (0.1, d), 241 (4, g), 239 (4, g), 164 (7, i), 136 (13, AH_2), 135 (100, AH). Anal. Calcd for $C_{10}H_{14}N_5O_3Br$: C, 36.16; H, 4.25; N, 21.09. Found: C, 36.29; H, 4.27; N, 21.11.

Preparation of 2'-iodo-2'-deoxyadenosine$_{ox\text{-}red}$ (51). A solution of 260 mg (0.5 mmole) of 42 and 320 mg (2.1 mmole) of potassium iodide in 40 ml of dry DMF was stirred at 120° for two hours. The solution was evaporated to a gum which was dissolved in a mixture of water and methylene chloride. The organic layer was extracted twice with sodium bicarbonate solution, twice with 10% sodium bisulfite solution, and twice with water. The combined

aqueous layers were extracted continuously overnight with ethyl acetate. This ethyl acetate layer was combined with the methylene chloride layer from above and the mixture evaporated to dryness. The residue was dissolved in ethanol/trace acetic acid and this solution was refluxed for several hours. It was then evaporated to dryness and the residue was dissolved and stirred in methanolic ammonia (pH 9) for one hour. This solution was evaporated to dryness and the residue was chromatographed on a silica gel column (100 ml) eluting with 10% methanol/methylene chloride. The product was crystallized from ethyl acetate to give 89 mg (43%) of 51 in three crops: mp 170-172°C; UV (H_2O) max 260 nm (ε 15,400); UV (1 M HCl) max 260 nm (ε 14,100); UV (1 M NaOH) max 260 nm (ε 15,800); NMR (DMSO-d_6) δ3.1-3.6 (unresolved, 5, CH_2 3', CH 4', and CH_2 5'), 3.90 (m, 2, CH_2 2'), 4.46 (t, 1, J = 5Hz, OH 5'), 4.72 (t, 1, J = 5Hz, OH 3'), 6.10 (dd, 1, J - 5Hz, J = 7Hz, CH 1'), 7.25 (s, 2, NH_2), 8.16 (s, 1, H2), 8.36 (s, 1, H8); ms (200°) m/e 379.0117 (calcd for M^+ 379.0141, 3%), 349 (0.2, d), 288 (3, g), 164 (4, i), 136 (19, AH_2), 135 (100, AH).

Anal. Calcd for $C_{10}H_{14}N_5O_3I$: C, 31.68; H, 3.72; N, 18.47. Found: C, 31.91; H, 3.77; N, 18.21.

Preparation of 2'-deoxyadenosine$_{ox\text{-}red}$ (53). To a solution of 25 mg (0.075 mmole) of 50 and 17 mg (0.30 mmole) of

potassium hydroxide in 15 ml of methanol was added 25 mg 5% palladium on carbon. The mixture was stirred in a stainless steel bomb under 45 psi hydrogen gas at room temperature for 16 hours. The mixture was filtered through a sintered glass funnel (porosity E) by suction, through paper by gravity, and through a layer of Celite by suction. The filtrate was evaporated to a gum which was chromatographed on a column of Dowex 1 x 2 hydroxide form, eluting with water. Evaporation of the appropriate fractions gave a white solid which was crystallized from ethyl acetate to give 11 mg (58%) of 53: mp 152.5-154.5°C; UV (H_2O) max 260 nm (ε 15,100); UV (1 M NaOH) max 260 nm (ε 15,700); NMR (DMSO-d_6) δ1.70 (d, 3, J = 6Hz, CH_3), 3.1-3.7 (unresolved and overlapped with H_2O, CH_2 3', CH 4', and CH_2 5'), 4.5 (br, 2, OH 3' and OH 5'), 6.08 (q, 1, J = 6Hz, CH 1'), 7.22 (s, 2, NH_2), 8.16 (s, 1, H2), 8.33 (s, 1, H8); ms (200°) m/e 253.1178 (calcd for M^+ 253.1175, 3%), 223 (1, d), 164 (2, i), 162 (30, g), 136 (20, AH_2), 135 (100, AH).

Anal. Calcd for $C_{10}H_{15}N_5O_3$: C, 47.42; H, 5.97; N, 27.66. Found: C, 47.44; H, 5.90; N, 27.49.

Preparation of 2'-amino-2'-deoxyadenosine$_{ox\text{-}red}$ (55). To a solution of 400 mg (1.36 mmole) of 48 in 90 ml of ethanol was added 2.0 g of a suspension of Raney nickel in water and 4.5 ml of 85% hydrazine hydrate in water. This mixture was stirred under nitrogen for two hours. The mixture

was filtered three times through paper by gravity. The filtrate was evaporated to a gum which was crystallized from 18 ml of absolute ethanol by ether diffusion to give 185 mg (51%) of crystalline 55 in two crops: mp 143-144.5°C; UV (H_2O) max 260 nm (ε 16,900); UV (1 M HCl) max 256 nm (ε 17,200); UV (1 M NaOH) max 260 nm (ε 17,600); NMR (DMSO-d_6) δ2.6-3.7 (unresolved, 12, NH_2 2', CH_2 2', OH 3', CH_2 3', CH 4', OH 5', and CH_2 5'), 5.79 (t, 1, J = 5Hz, CH 1'), 7.20 (s, 2, NH_2), 8.14 (s, 1, H2), 8.27 (s, 1, H8); ms (150°) m/e 269.1375 (calcd for M+1$^+$ 269.1327, 0.4%), 177 (9, g), 164 (100, i), 136 (51, AH_2), 135 (33, AH). Anal. Calcd for $C_{10}H_{16}N_6O_3$: C, 44.77; H, 6.01; N, 31.33. Found: C, 44.71; H, 5.77; N, 31.43.

Preparation of 2'-N-phenylalanyl-2'-amino-2'-deoxyadenosine$_{ox\text{-}red}$ (56).

A solution of 50 mg (0.19 mmole) of 55 in 2 ml methanol was mixed with a solution of 160 mg (0.38 mmole) of the p-nitrophenyl ester of N-carbobenzoxyphenylalanine in 4 ml dichloromethane. The resulting yellow solution was stirred overnight at room temperature, then evaporated to a gum which was chromatographed on a column of silica gel (17 g) eluting with 200 ml 10% methanol/methylene chloride then with 100 ml 20% methanol/methylene chloride. The appropriate fractions were evaporated to a glass. This residue was dissolved in methanol and stirred overnight with 70 mg 5% palladium on carbon under 45 psi hydrogen gas.

The catalyst was removed by filtration through paper and the filtrate reduced to a small volume. Crystallization from this solution by ether diffusion gave 9 mg (12%) of 56: mp 143-150°C; UV (H_2O) max 260 nm (ε 13,400); UV (1 M HCl) max 257 nm (ε 13,800); UV (1 M NaOH) max 260 nm (ε 13,700); NMR (DMSO-d_6) δ1.55 (v br, 2, NH_2 phenylalanyl), 2.37 (m, overlap with DMSO, C<u>H</u>-NH_2 phenylalanyl), 2.68 (d, 1, J = 4 Hz, C<u>H</u>-Ar phenylalanyl), 2.84 (d, 1, J = 4 Hz, C<u>H</u>-Ar phenylalanyl), 3.23 (br, CH 4', $CH_2$5'), 3.49 (br, CH_2 3'), 3.74 (br t, 2, CH_2 2'), 4.48 (br, 1, OH 5'), 4.75 (br, 1, OH 3'), 5.96 (t, 1, J = 6 Hz, CH 1'), 7.13 (s, 2, NH_2), 7.18 (m, 5, CH aromatic), 8.08 (br, 1, NH 2'), 8.14 (s, 1, H_2), 8.23 (s, 1, H8).

Anal. Calcd for $C_{19}H_{25}N_7O_4$: C, 54.93; H, 6.07; N, 23.60. Found: C, 54.73; H, 6.03; N, 23.41.

<u>Preparation of 2'-N-leucyl-2'-amino-2'-deoxyadenosine</u>$_{ox\text{-}red}$ (<u>57</u>)

This compound was prepared and purified by the same procedure used for <u>56</u> using 100 mg (0.38 mmole) of <u>55</u> and 288 mg (0.76 mmole) of the p-nitrophenyl ester of N-carbobenzoxyleucine. Crystallization from absolute ethanol by ether diffusion gave 38 mg (27%) of <u>57</u>: mp 190-192.5°C; NMR (DMSO-d_6) δ0.70 (d, 6, J = 6Hz, CH_3, CH_3), 1.05 (dd, 2, J = 6Hz, J = 6Hz, CH_2 leucyl), 1.42 (m, 1, CH leucyl), 3.00 (m, 1, C<u>H</u>-NH_2), 3.20 (m, CH 4', CH_2 5'), 3.30 (br, NH_2 leucyl), 3.50 (br, 2, CH_2 3'), 3.71 (br, 2,

CH_2 2'), 4.42 (br, 1, OH 3'), 5.96 (t, 1, J = 6Hz, CH 1'), 7.18 (s, 2, NH_2), 8.00 (br, 1, NH 2'), 8.12 (s, 1, H2), 8.22 (s, 1, H8).

Anal. Calcd for $C_{16}H_{27}N_7O_4$: C, 50.38; H, 7.14; N, 25.71. Found: C, 49.98; H, 7.10; N, 25.35.

Preparation of 1',2'-unsaturated 2'-deoxyadenosine$_{ox\text{-}red}$ (54).

A 246 mg (0.57 mmole) sample of 47 was deblocked in the usual way and the product was chromatographed on a column of Dowex 1x2 hydroxide form, eluting with water. The appropriate fractions were evaporated to dryness and the residue crystallized from ethanol by ether diffusion to give 33 mg (23%) of 54: mp 197-198°C; NMR (DMSO-d_6) δ3.65 (t, 4, J = 5Hz, CH_2 3', CH_2 5'), 4.28 (m, 1, CH 4'), 4.50 (d, 1, J = 3Hz, CH 2'), 5.10 (t, 2, J = 6Hz, OH 3', OH 5'), 5.12 (d, 1, J = 3Hz, CH 2'), 7.40 (s, 2, NH_2), 8.20 (s, 1, H2), 8.39 (s, 1, H8); ms (200°) m/e 251.1022 (calcd for M^+ 251.1026, 1, M), 221 (9, d), 136 (28, AH_2), 135 (100, AH).

Anal. Calcd for $C_{10}H_{13}N_5O_3 \cdot 0.25\ H_2O$: C, 46.96; H, 5.32; N, 27.39 Found: C, 47.09; H, 5.05; N, 26.96.

Preparation of 5'-O-tosyladenosine (60).

4.5 g (9.8 mmole) 2',3'-Isopropylidene-5'-O-tosyl-adenosine (59) was deblocked by dissolving in 90 ml of 90% trifluoroacetic acid and allowing the solution to stand for 15 minutes at room temperature. The solution was

evaporated to a gum, which was repeatedly evaporated from water then repeatedly evaporated from absolute ethanol to give a white foam. This material was chromatographed on a silica gel column (3.75 cm x 45 cm) eluting with 10% methanol/methylene chloride. Evaporation of the appropriate fractions gave a white foam which crystallized from methanol to give 2.38 g (58%) of 60 in five crops: mp 154-156°C (lit. 155-156°C); UV (1 M HCl) max 261 nm (ε 14,800); NMR (DMSO-d_6) δ2.40 (s, 3, CH_3), 4.15 (m, 2, CH 3', CH 4'), 4.30 (d, 2, J = 4Hz, CH_2 5'), 4.60 (m, 1, CH 2'), 5.35 (d, 1, J = 5Hz, OH 3'), 5.53 (d, 1, J = 6Hz, OH 2'), 5.88 (d, 1, J = 5Hz, CH 1'), 7.25 (s, 2, NH_2), 7.33 (d, 2, J = 8Hz, CH aromatic), 7.75 (d, 2, J = 8Hz, CH aromatic), 8.10 (s, 1, H2), 8.20 (s, 1, H8).
Anal. Calcd for $C_{17}H_{19}N_5O_6S$: C, 48.45; H, 4.54; N, 16.62. Found: C, 48.19, H, 4.53; N, 16.56.

Preparation of 5'-O-tosyladenosine$_{ox\text{-}red}$ (61).

The oxidation-reduction was carried out as before using 500 mg (1.2 mmole) 60, 280 mg (1.3 mmole) sodium periodate, and 100 mg (2.6 mmole) sodium borohydride. For solubility the reaction was carried out in 100 ml of 3:1 water/DMF. After neutralization the solution was evaporated to dryness. The residue was swirled with 15 ml of 15% methanol/methylene chloride and the insoluble salts removed by filtration. The material in the filtrate

was chromatographed on a silica gel column (75 ml) eluting with 15% methanol/methylene chloride. Evaporation of the appropriate fractions gave a gum. This was dissolved in methanol and on standing in the refrigerator 365 mg (75%) of 61 was collected as a white powder: mp 164-168°C; UV (1 M HCl) max 260 nm (ε 13,900); NMR (DMSO-d_6) δ2.38 (s, 3, CH_3), 3.50 (m, 3, CH_2 3', CH 4'), 3.84 (m, 4, CH_2 2', CH_2 5'), 4.90 (t, 1, J = 5Hz, OH 3'), 5.15 (t, 1, J = 5.5Hz, OH 2'), 5.76 (t, 1, J = 5.5Hz, CH 1'), 7.14 (s, 2, NH_2), 7.32 (d, 2, J = 8Hz, CH aromatic), 7.51 (d, 2, J = 8Hz, CH aromatic), 8.10 (s, 1, H2), 8.13 (s, 1, H8); ms (140°) m/3 252 (2, k), 178 (11, h), 164 (11, i), 136 (23, AH_2), 135 (100, AH).

Anal. Calcd for $C_{17}H_{21}N_5O_6S$: C, 48.22; H, 5.00; N, 16.54. Found: C, 47.93; H, 5.00; N, 16.37.

Preparation of 5'-azido-5'-deoxyadenosine (62).

A solution of 2.0 g (4.75 mmole) of 60 and 1.2 g (24 mmole) lithium azide in 50 ml dry DMF was stirred at 80°C for 45 minutes. The solution was evaporated to give a white foam. This was crystallized from water to give 771 mg (56%) of 62 as fine white needles: mp 115-121°C (lit. no sharp mp); UV (H_2O) max 260 nm (ε 15,000); UV (1 M HCl), max 258 nm (ε 14,800); UC (1 M NaOH) max 260 nm (ε 15,100); NMR (DMSO-d_6) δ3.61 (m, 2, CH_2 5'), 4.05 (m, 1, CH 4'), 4.11 (t, 1, J = 5Hz, CH 3'), 4.75 (t, 1, J = 5Hz), CH 2'), 5.46 (br s, 2, OH 2', OH 3'), 5.93 (d, 1,

J = 5Hz, CH 1'), 7.27 (s, 2, NH_2), 8.18 (s, 1, H2), 8.36 (s, 1, H8); ms (150-200°) m/e 292.1035 (calcd for M^+ 292.1027, 6, M), 250 (10, j), 178 (88, h), 164 (63, i), 136 (100, AH_2), 135 (94, AH).

Anal. Calcd for $C_{10}H_{12}N_8O_3 \cdot 0.25\ H_2O$: C, 40.47; H, 4.24; N, 37.76. Found: C, 40.46; H, 4.63; N, 37.70.

Preparation of 5'-azido-5'-deoxyadenosine$_{ox\text{-}red}$ (63).

The oxidation-reduction was carried out accoring to the standard procedure starting with 250 mg (0.86 mmole) of 62. After neutralization the solution was evaporated to a gum which was chromatographed on a column of Dowex 1x2 hydroxide form (75 ml) eluting with water. The appropriate fractions were evaporated to a gum which was crystallized from methanol to give 134 mg (54%) of 63 in two crops: UV (H_2O) max 260 nm (ε 15,200); UV (1 M HCl) max 259 (ε 14,900); UV (1 M NaOH) max 260 nm (ε 15,100); NMR (DMSO-d_6) δ3.16 (d, 2, J = 5Hz, CH_2 5'), 3.56 (overlapped with H_2O, CH_2 3', CH 4'), 3.96 (br d, 2, CH_2 2'), 4.94 (br, 1, OH 3'), 5.21 (br, 1, OH 3'), 5.21 (br, 1, OH 2'), 5.89 (t, 1, J = 6Hz, CH 1'), 7.26 (s, 2, NH_2), 8.19 (s, 1, H2), 8.30 (s, 1, H8); ms (150°) m/3 294.1200 (calcd for M^+ 294.1189, 3, M), 252 (5, k), 178 (2, h), 164 (15, i), 136 (37, AH_2), 135 (100, AH).

Anal. Calcd for $C_{10}H_{14}N_8O_3$: C, 40.81; H, 4.79; N, 38.08. Found: C, 40.81; H, 4.85; N, 38.25.

Preparation of 5'-chloro-5'-deoxyadenosine (64).

To 1.34 g (5 mmole) adenosine suspended in a mixture of 15 ml dry acetonitrile and 791 mg (10 mmole) dry pyridine was added 3.0 g (25 mmole) thionyl chloride dropwise with stirring. The nucleoside dissolved in a few minutes. After stirring overnight a white precipitate had formed. This was dissolved in 20 ml 9:1 methanol/conc ammonia and white crystals formed after a few minutes. This material was recrystallized from water to give 996 mg (70%) of 64 as fine white needles: mp 107-110°C; UV (H_2O) max 260 nm (ε 15,400); UV (1 M HCl) max 257 nm (ε 15,300); UV (1 M NaOH) max 260 nm (ε 15,900); NMR (DMSO-d_6) δ3.91 (d, 2, J = 4Hz, CH_2 5'), 4.15 (m, 2, CH 3', CH 4'), 4.96 (m, 1, CH 2'), 5.43 (d, 1, J = 5Hz, OH 3'), 5.57 (d, 1, J = 6Hz, OH 2'), 5.94 (d, 1, J = 5Hz, CH 1'), 7.27 (s, 2, NH_2), 8.17 (s, 1, H2), 8.34 (s, 1, H8); ms (150-200°) m/e 287.0602 (calcd for M^+ 287.0599, 3, M), 285.0625 (calcd for M^+ 285.0639, 8, M), 178 (5, h), 164 (91, i), 136 (100, AH_2), 135 (83, AH).

Anal. Calcd for $C_{10}H_{12}N_5O_3Cl$: C, 42.04; H, 4.24; N, 24.52. Found: C, 42.22; H, 4.52; N, 24.68.

Preparation of 5'-chloro-5'deoxyadenosine$_{ox\text{-}red}$ (65).

Oxidation-reduction was carried out using the standard procedure, starting with 1.0 g (3.5 mmole) of 64. After neutralization the volume of the reaction solution was increased to 500 ml. The solution was stirred with 10 g

charcoal for one hour. The carbon was packed in a column and washed with 2 litres of water. The nucleoside was eluted with 1 litre 50% acetonitrile/water which was then evaporated to give a white foam. Crystallization from methanol/methylene chloride gave 613 mg (61%) of 66 in three crops: mp 75-78°C; UV (H_2O) max 260 nm (ε 14,800); UV (1 M HCl) max 258 nm (ε 14,600); UV (1 M NaOH) max 261 nm (ε 15,100); NMR (DMSO-d_6) δ3.3-3.8 (unresolved, 5, CH_2 3', CH 4', CH_2 5'), 3.92 (t, 2, J = 6Hz, CH_2 2'), 4.93 (t, 1, J = 6Hz, OH 3'), 5.16 (t, 1, J - 6Hz, OH 2'), 5.84 (t, 1, J = 6Hz, CH 1'), 7.20 (s, 2, NH_2), 8.14 (s, 1, H2), 8.27 (s, 1, H8); ms (150°) m/e 287.0789 (calcd for M^+ 287.0774, 4, M), 252 (8, k), 179 (20, h), 164 (22, i), 136 (40, AH_2), 135 (100, AH).

Preparation of 5'-amino-5'-deoxyadenosine (66).

To 240 mg (0.82 mmole) 62 in 45 ml ethanol was added 750 mg of a suspension of Raney nickel in water and 2.25 ml 85% hydrazine hydrate in water. This mixture was stirred under nitrogen for two hours. The mixture was filtered through paper by gravity several times. The filtrate was evaporated to dryness then the residue was crystallized from ethanol by ether diffusion to give 110 mg (42%) of 67 in two crops: mp 190-196°C with decomposition (literature reports HCl salt only); UV (H_2O) max 260 nm (ε 14,600); UV (1 M HCl) max 258 nm (ε 14,800), UV (1 M NaOH) max 260 nm (ε 14,900); NMR (DMSO-d_6) δ2.77

(d, 2, J = 4Hz, CH_2 5'), 2.6-3.6 (br, NH_2 5'), OH 2', OH 3'), 3.85 (m, 1, CH 4'), 4.14 (br t, 1, J - 5Hz, CH 3'), 4.66 (t, 1, J = 6Hz, CH 2'), 5.84 (d, 1, J = 6Hz, CH 1'), 7.14 (s, 2, NH_2), 8.12 (s, 1, H2), 8.35 (s, 1, H8); ms (150-200°) m/e 267.1207 (calcd for M+1 267.1200, 1, M+1), 266 (1, M), 237 (22, j), 178 (80, h), 164 (35, i), 136 (100, AH_2), 135 (49, AH).

Anal. Calcd for $C_{10}H_{14}N_6O_3$: C, 45.11, H, 5.30; N, 31.57. Found: C, 44.91; H, 5.31; N, 31.03.

Preparation of 5'-amino-5'-deoxyadenosine$_{ox\text{-}red}$ (67).

To a solution of 300 mg (1 mmole) 63 in 65 ml methanol was added 900 mg of a suspension of Raney nickel in water and 3.5 ml of 85% hydrazine hydrate in water. The mixture was stirred under nitrogen for two hours. The mixture was then filtered through paper by gravity several times and the final fitrate evaporated to a white foam. Crystallization from methanol by ether diffusion gave 151 mg (51%) of 67 in two crops: mp 149-152°C; UV (H_2O) max 259 nm (ε 15,600); UV (1 M HCl) max 256 nm (ε 15,100); UV (1 M NaOH) max 259 nm (ε 15,800); NMR (DMSO-d_6) δ2.6-3.6 (v br, OH 2', OH 3', NH_2 5'), 3.27 (m, 1, CH 4'), 3.50 (d, 2, J = 5Hz, CH_2 3'), 3.89 (d, 2, J = 6Hz, CH_2 2'), 5.86 (t, 1, J = 6Hz, CH 1'), 7.21 (s, 2, NH_2), 8.15 (s, 1, H2), 8.32 (s, 1, H8); ms (150-200°) m/e 269.1362 (calcd for M+1 269.1362, 2, M+1), 268 (1, M), 239 (22, d), 178 (57,

h), 164 (30, i), 136 (100, AH_2), 135 (89, AH).

Anal. Calcd for $C_{10}H_{16}N_6O_3$: C, 44.77; H, 6.01; N, 31.33. Found: C, 44.89; H, 6.08; N, 31.27.

Preparation of 5'-deoxyadenosine (68).

To a solution of 500 mg (1.75 mmole) 64 in 25 ml dry DMSO was added 25 ml 1 M lithium triethylborohydride ("superhydride") (25 mmole) in THF. The solution was stirred under argon at room temperature for three days. 25 ml 10% acetic acid was added dropwise to destroy excess hydride. The solution was evaporated exhaustively, first on a rotary evaporator equipped with a water cooled condenser then on one with a dry ice condenser. The water cooled condenser was used initially so that the volatile flammable byproduct triethyl borane passes into the vacuum line without condensing. Even after exhaustive evaporation traces of triethyl borane sometimes burst into flame. The residue was chromatographed on a column of Dowex 1x2 hydroxide form (100 ml) eluting first with water, then with 80% methanol water. The product was crystallized from water giving 279 mg (64%) 68 in three crops: mp 216-218°C with a solid-solid transition at 135-140°; UV (H_2O) max 260 nm (ε 14,300); UV (1 M HCl) max 257 nm (ε 14.800); UV (1 M NaOH) max 259 nm (ε 15,000); NMR (DMSO-d_6) δ1.31 (d, 3, J = 6Hz, CH_3), 3.98 (m, 2, CH 3', CH 4'), 4.66 (m, 1, CH 2'), 5.14 (d, 1, J = 5Hz, OH 3'), 5.42 (d, 1, J = 6Hz, OH 2'), 5.85 (d, 1, J = 6Hz, CH 1'), 7.25 (s, 2, NH_2),

8.17 (s, 1, H2), 8.32 (s, 1, H8); ms (150-200°) m/e 251.1010 (calcd for M^+ 251.1043, 15, M), 178 (26, h), 164 (100, i), 136 (98, AH_2), 135 (92, AH).

Anal. Calcd for $C_{10}H_{13}N_5O_3$: C, 47.80; H, 5.21; N, 27.88. Found: C, 48.14; H, 5.41; N, 27.55.

Preparation of 5'-deoxyadenosine$_{ox\text{-}red}$ (69).

The oxidation-reduction procedure was carried out as before starting with 57 mg (0.21 mmole) 5'-deoxyadenosine. After neutralization the volume of the solution was reduced for chromatography, but further purification was unnecessary as 32 mg (56%) of 69 crystallized from the reaction mixture: UV (H_2O) max 260 nm (ε 13,600); UV (1 M HCl) max 256 nm (ε 13,000); UV (1 M NaOH) max 260 nm (ε 14,400); NMR (DMSO-d_6) δ0.76 (d, 3, J = 6Hz, CH_3), 3.43 (overlapped with H_2O, CH_2 3'), 3.56 (m, 1, CH 4'), 3.88 (t, 2, J = 6Hz, CH_2 2'), 4.73 (t, 1, J = 6Hz, OH 3'), 5.12 (t, 1, J = 6Hz, OH 2'), 5.84 (t, 1, J = 6Hz, CH 1'), 7.22 (s, 2, NH_2), 8.16 (s, 1, H2), 8.26 (s, 1, H8); ms (150-200°) m/e 253.1170 (calcd for M^+ 253.1189, 10, M), 222 (19, 3), 178 (27, h), 164 (25, i), 136 (48, AH_2), 135 (100, AH).

Anal. Calcd for $C_{10}H_{15}N_5O_3$: C, 47.42; H, 5.97; N, 27.66. Found: C, 47.20; H, 6.20; N, 27.41.

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APPENDIX

Kinetic Data

The methods used are described in the experimental section. Unless indicated otherwise the temperature was 50°C and the rate constants and correlation coefficients were determined by the least squares programme.

Hydrolysis of Adenosine (1)

time (min)	% nucleoside remaining
15	93,91,88
30	86,83,76
45	76,78,67
60	73,67,57
90	60,52,51
120	49,51,37
170	35,38,25
220	27,22,17
270	19,14,12

$k = 6.22\ (\pm.09),\ 7.10\ (\pm.35),\ 7.44\ (\pm.19) \times 10^{-3}\ min^{-1}$

$r = .999,\ .992,\ .998$

Hydrolysis of $Adenosine_{ox\text{-}red}$ (25)

time (min)	% nucleoside remaining
2,2,1	82,76,88
4,4,3	65,60,74
6,6,5	58,48,60
8,8,7	48,41,54
-,10,9	-,32,40
15,15,12	25,21,30
20,20,15	16,15,]1
30,25,18	6,7,17
-,-,21	-,-,13

$k = 9.25\ (\pm.12),\ 9.75\ (\pm.40),\ 9.83\ (\pm.23) \times 10^{-2}\ min^{-1}$

$r = .99,\ .995,\ .998$

At 0°C

time (hr)	% nucleoside remaining
25	95,94
66	84,85
75	85,83
89	78,82
98	79,77
115	77,75
138	74,71
164	67,71
185	67,65

$k = 2.20\ (\pm.16),\ 2.20\ (\pm.15) \times 10^{-3}\ hr^{-1}$

$r = .981,\ .984$

Hydrolysis of 2'-0-tosyladenosine$_{ox\text{-}red}$ (43)

time (hr)	% nucleoside remaining
3	87,89,82
5	57,85,87
8	77,72,71
13	51,49,52
20	34,42,29
22	29,25,23
24	25,24,17
30	17,21,13
33	16,17,13

$k = 5.60\ (\pm.44),\ 5.75\ (\pm.42),\ 7.11\ (\pm.43) \times 10^{-2}\ hr^{-1}$

$r = .979,\ .982,\ .988$

Hydrolysis of 2'-azido-2'-deoxyadenosine$_{ox\text{-}red}$ (48)

time(min)	% nucleoside remaining
15,10,12	80,88,81
30,20,20	64,69,81
45,30,32	50,65,66
60,45,45	39,53,60
90,60,60	23,41,45
120,75,75	10,28,41
170,90,90	8,29,50
220,120,120	4,17,21
-,150,150	-,13,15

$k = 1.62\ (\pm.14),\ 1.38\ (\pm.07),\ 1.27\ (\pm.04) \times 10^{-2}\ min^{-1}$

$r = .981,\ .992,\ .997$

Hydrolysis of 2'-chloro-2'-deoxyadenosine$_{ox\text{-}red}$ (49)

time (hr)	% nucleoside remaining
0.5,0.5,0.5	86,92,90
1,1,1	78,76,80
2,1.5,1.5	60,67,70
3,2.8,2	47,52,61
4,3,2.5	39,48,50
4.5,3.8,3	25,32,49
5.8,4,4	18,36,31
6.5,4.5,4.5	12,32,26
7.3,5,-	13,25,-

$k = 3.08\ (\pm.19),\ 2.76\ (\pm.16),\ 3.10\ (\pm.16) \times 10^{-1}\ hr^{-1}$

$r = .986,\ .988,\ .992$

Hydrolysis of 2'-bromo-2'-deoxyadenosine (50)

time(hr)	% nucleoside remaining
0.5,0.5,0.5	83,83,80
1,1,1	64,67,61
1.5,1.5,1.5	51,59,54
2,2,2	43,42,41
2.5,3,2.5	32,47,36
3.3,3.3,3	21,26,28
3.5,3.5,3.5	22,24,22
4.8,4,4	13,19,21
-,4.3,4.5	-,17,13

$k = 4.40\ (\pm.14),\ 4.24\ (\pm1.0),\ 4.21\ (\pm.21) \times 10^{-1}\ hr^{-1}$

$r = .997,\ .998,\ .992$

Hydrolysis of 2'-iodo-2'-deoxyadenosine$_{ox\text{-}red}$ (51) at 0°C

time (hr)	% nucleoside remaining
25	89,89,89
66	69,69,73
75	66,69,67
89	61,62,63
98	62,58,59
115	55,46,61
138	47,53,46
164	39,43,44
185	40,40,41

$k = 5.28\ (\pm.31),\ 4.94\ (\pm.54),\ 4.97\ (\pm.35) \times 10^{-3}\ hr^{-1}$

$r = .988,\ .960,\ .982$

Hydrolysis of 2'-deoxyadenosine$_{ox-red}$ (53) at 0°C

time (min)	% nucleoside remaining
10	84,87,85
20	81,79,80
30	71,73,73
40	65,62,63
50	59,60,58
60	59,54,48
70	49,48,49
80	44,39,44
90	38,37,40

$k = 9.74\ (\pm.58),\ 10.9\ (\pm.52),\ 9.80\ (\pm.54) \times 10^{-3}\ min^{-1}$

$r = .988,\ .992,\ .989$

Hydrolysis of 4-(9-adeninyl)-2-hydroxymethyl-3-oxapent-4-en-1-ol (54)

time (min)	% nucleoside remaining
2	68,75,73
4	60,60,60
6	50,50,50
8	41,44,39
10	44,37,35
12	25,33,28
14	23,30,22
16	20,22,17
18	14,18,24

$k = 9.72\ (\pm.78),\ 8.36\ (\pm.37),\ 10.2\ (\pm.31) \times 10^{-2}\ min^{-1}$

$r = .978,\ .993,\ .997$

Hydrolysis of 2'-amino-2'-deoxyadenosine$_{ox\text{-}red}$ (55)

time (hr)	% nucleoside remaining
1,0.5,0.5	76,87,88
2,1,1	58,79,79
4,1.5,1.5	34,72,64
6,2,2	18,58,54
11,3,3	3,46,46
-,4,4	-,36,39
-,4.5,5	-,32,32
-,6,6	-,22,25
-,8,8	-,12,16

$k = 2.87\ (\pm.09),\ 2.66\ (\pm.07),\ 2.42\ (\pm.15) \times 10^{-1}\ hr^{-1}$

$r = .999,\ .998,\ .986$

Hydolysis of 5'-O-tosyladenosine (60)

time (hr)	% nucleoside remaining
8	79,78,73
23	61,50,41
26	56,52,45
31	44,38,37
48	27,17,30
51	32,15,24
55	31,10,21
58	4,9,12

$k = 3.92\ (\pm1.36),\ 4.51\ (\pm.34),\ 2.85\ (\pm.43) \times 10^{-2}\ hr^{-1}$

$r = .763,\ .983,\ .937$

Hydrolysis of 5'-O-tosyladenosine$_{ox\text{-}red}$ (61)

time (hr)	% nucleoside remaining
0.5	9.94,93
1	85,90,86
1.5	77,77,79
2	70,72,70
2.5	66,63,71
3	61,59,61
3.5	58,51,56
4	50,49,50
4.5	52,46,-

$k = 1.51\ (\pm.09),\ 1.90\ (\pm.08),\ 1.72\ (\pm.10) \times 10^{-1}\ hr^{-1}$

$r = .988,\ .993,\ .990$

Hydrolysis of 5'-azido-5'-deoxyadenosine (62)

time (hr)	% nucleoside remaining
3	87,92,91
5	84,81,85
8	69,74,68
13	58,59,53
20	42,26,34
22	40,35,40
24	32,30,34
30	26,22,22
33	16,14,16

$k = 5.13\ (\pm.37),\ 5.92\ (\pm.50),\ 5.39\ (\pm.35) \times 10^{-2}\ hr^{-1}$

$r = .982,\ .976,\ .986$

Hydrolysis of 5'-azido-5'-deoxyadenosine$_{ox\text{-}red}$ (63)

time (hr)	% nucleoside remaining
0.3	77,86,86
0.5	72,75,79
0.8	59,61,59
1	53,51,51
1.3	41,46,46
1.5	34,45,35
2	22,28,27
2.5	17,18,20
3	14,13,14

$k = 6.83\ (\pm.28),\ 6.86\ (\pm.29),\ 6.62\ (\pm.19) \times 10^{-1}\ hr^{-1}$

$r = .994,\ .993,\ .997$

Hydrolysis of 5'-chloro-5'-deoxyadenosine (64)

time (hr)	% nucleoside remaining
0.5,-,2	99,-,93
1,-,4	97,-,91
2,-,6	94,-,85
3,-,8	90,-,78
4,4,10	86,91,78
5.5,-,10	84,-,81
11,8,26	69,81,44
24,22,26	41,54,49
29,25,-	32,44,-
35,-,-	40,-,-

$k = 3.22\ (\pm.29),\ 3.28\ (\pm.30),\ 3.00\ (\pm.19) \times 10^{-2}\ hr^{-1}$

$r = .969,\ .991,\ .988$

Hydrolysis of 5'-chloro-5'deoxyadenosine$_{ox\text{-}red}$ (65)

time (hr)	% nucleoside remaining
0.5	78,84,77
1	57,63,69
1.5	49,55,45
2	43,39,39
2.5	27,32,36
3	25,23,29
3.5	21,21,20
4	13,15,14
4.5	9,12,14

$k = 5.10\ (\pm.31),\ 4.85\ (\pm.14),\ 4.51\ (\pm.28) \times 10^{-1}\ hr^{-1}$

$r = .987,\ .997,\ .987$

Hydrolysis of 5'-amino-5'-deoxyadenosine (66)

time (hr)	% nucleoside remaining
3.5	91,90,93
7.5	90,89,87
10	84,86,88
11	89,86,90
23.5	82,73,76
25	73,75,76
29.5	77,78,76
34.5	77,71,61
47.5	63,63,76
53	52,57,58

$k = 8.33,\ 7.59,\ 8.89 \times 10^{-3}\ hr^{-1}$ (graphic analysis)

Hydrolysis of 5'-amino-5'-deoxyadenosine$_{ox\text{-}red}$ (67)

time (hr)	% nucleoside remaining
0.5	93,93,90
1	84,87,85
1.5	79,82,77
2	73,81,68
2.5	73,67,71
3	63,71,63
3.5	56,52,53
4	54,62,57
4.5	52,52,48

$k = 1.50, 1.45, 1.50 \times 10^{-1}$ hr^{-1} (graphic analysis)

Hydrolysis of 5'-deoxyadenosine (68)

time (min)	% nucleoside remaining
6	89,85,84
12	78,70,74
18	61,62,59
24	52,55,56
30	43,50,54
36	41,39,33
42	29,36,31
48	27,25,26
54	29,22,20

$k = 2.61\ (\pm.21),\ 2.76\ (\pm.16),\ 2.97\ (\pm.22) \times 10^{-2}$ min^{-1}

$r = .978,\ .988,\ .981$

Hydrolysis of 5'-deoxyadenosine$_{ox\text{-}red}$ (69) at 0°C

time(hr)	% nucleoside remaining
5	92,91,92
19	73,73,77
21	75,72,74
24	74,65,67
29	67,65,61
43	55,53,48
45	47,57,49
50	55,53,50
52	48,54,49

$k = 1.35, 1.17, 1.44 \times 10^{-2}$ hr^{-1} (graphic analysis)